Plant health

Nanocrystals spraying interval for the control of tomato bacterial spot caused by *Xanthomonas hortorum* pv. *gardneri*[1]

Ludyellen Cristina Medeiros Santos[2], *Anielle Christine Almeida Silva*[3], *Nilvanira Donizete Tebaldi*[2*]

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ABSTRACT

Tomato bacterial spot caused by *Xanthomonas hortorum* pv. *gardneri* triggers significant losses in crop production, and the active ingredients availability for disease control is limited. For this reason, there is a great demand for plant protection alternatives, such as the use of nanocrystals. Thus, the aim of this work was to evaluate the performance of nanocrystals spraying intervals for the control of tomato bacterial spot. Tomato plants of cv. Santa Cruz Kada were sprayed at 3-4 leaf stage under greenhouse conditions with ZnO:1Mg, ZnOCl, and ZnOCl:0.1Cu nanocrystals, copper and water. Three days later, the plants were inoculated with a bacterial suspension (10^9 CFU mL^{-1}). Then, after 3, 6, 9, or 12-day intervals, the plants were sprayed with the products. The bacterial spot severity was periodically quantified as affected leaf area percentage, and the area under the disease progress curve was calculated. Nanocrystals ZnO:1Mg, ZnOCl, and ZnOCl:0.1Cu reduced the tomato bacterial spot severity when sprayed at 3- and 6-day intervals. Thus, nanocrystals may be used for the tomato bacterial spot control when sprayed at 6-day intervals, once this interval is adequate and practical for disease management.

Keywords: disease; nanoparticle; severity; *Solanum lycopersicon*.

INTRODUCTION

Tomato bacterial spot, caused by four *Xanthomonas* species, *X. vesicatoria* (Jones *et al.*, 2004), *X. euvesicatoria* pv. *euvesicatoria, X. euvesicatoria* pv. *perforans* (Constantin *et al.*, 2016), and *X. hortorum* pv. *gardneri* (Morinière *et al.*, 2020), may cause significant production losses, especially under high moisture conditions and temperatures between 20 and 30 °C (Kurozawa & Pavan, 2005). In Brazil, *X. hortorum* pv. *gardneri* is located at regions with higher altitudes (900 m) and lower temperatures (20 °C), while *X. euvesicatoria* pv. *perforans* is predominant and widespread distributed around the country. The other two species, *X. vesicatoria* and *X. euvesicatoria* pv. *euvesicatoria*, have low occurrence in the field (Araújo *et al.*, 2017).

Controlling the disease is difficult due to fast spreads among plants under favorable conditions, is seed-born nature, and few registered chemical products (Nascimento *et al.*, 2013). Such as benzalkonium chloride, acibenzolar-S-methyl, laminarin (MAPA, 2022), antibiotics (kasugamycin), copper-based products, and copper carbamate mixtures are not always effective, and may select resistant strains (Itako *et al.*, 2012). In this scenario, nanoparticles or nanocrystals which are particles on a nanometric scale emerge as an innovative method to control phytopathogens in agriculture (Rai, 2013). Nanocrystals show low genotoxicity and high efficacy as biocides, due to their size, surface/volume ratio, and interaction with microorganism

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[2] Universidade Federal de Uberlândia, Instituto de Ciências Agrárias, Uberlândia, MG, Brazil. ludyellencristina@hotmail.com; nilvanira.tebdaldi@ufu.br
[3] Universidade Federal de Alagoas, Instituto de Física, Laboratório de Novos Materiais Nanoestruturados e Funcionais, Maceió, AL, Brazil. aniellechristineas@gmail.com
*Corresponding author: nilvanira.tebaldi@ufu.br

membranes (Allaker, 2010).

Zinc oxyde (ZnO) nanocrystals may be doped with different elements, such as copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), cobalt (Co), chromium (Cr), molybdenum (Mo), niobium (Nb), vanadium (V), ruthenium (Ru), silver (Ag), platinum (Pt) and gold (Au) (Zaleska, 2008), to increase their bactericidal effect. Doping is a process that consists of adding new elements to the nanoparticle's structure, and is also a form of adjusting the properties of functional oxides by altering their physical and electronic structure and changing their chemical characteristics (Callister Júnior, 2002).

The application of ZnO nanoformulations reduced bacterial canker in citrus, caused by *Xanthomonas citri* subsp. *citri* (Graham *et al.*, 2016), and maize white spot disease, caused by *Pantoea ananatis* (Mamede *et al.*, 2022). In tomato plants, the use of MgO nanoparticles increased the plant systemic resistance to *Ralstonia solanacearum* (Imada *et al.*, 2016). The incidence and severity of tomato bacterial spot caused by *X. euvesicatoria* pv. *perforans* were reduced with the use of Ag-dsDNA-GO nanocompound (Ocsoy *et al.*, 2013; Strayer *et al.*, 2016), and TiO_2 nanoparticles doped with Zn (Paret *et al.*, 2013). Also the use of ZnOCl, ZnOCl:0.1Ag, ZnOCl:1Cu (Oliveira *et al.*, 2023), ZnO:0.5Mo, ZnO:1K, and ZnO:1Mg (Fraga *et al.*, 2021) reduced the disease severity of tomato bacterial spot, caused by *X. hortorum* pv. *gardneri*.

However, to our acknowledgment, there are no references were about nanocrystals application intervals for the control of tomato bacterial spot caused by *X. hortorum* pv. *gardneri*. The intervals between spraying rounds may ensure the plant diseases control, to prevent economic damages to the crop, and also, the spraying intervals of essential oils in six days reduced the severity of tomato bacterial spot, caused by *X. hortorum* pv. *gardneri* (Araújo & Tebaldi, 2019). Based on this preliminary information, the aim of this work was to evaluate the performance of nanocrystals (ZnO:1Mg, ZnOCl, and ZnOCl:1Cu) spraying intervals for the control of tomato bacterial spot, caused by *X. hortorum* pv. *gardneri*.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse and in the Laboratório de Bacteriologia de Plantas (LABAC) of the Universidade Federal de Uberlândia (UFU) - Instituto de Ciências Agrárias, MG, in July 2019.

The *X. hortorum* pv. *gardneri* strain UFU A35 (Copper-sensitive), preserved and maintained at the LABAC's collection, was grown on medium 523 (Kado & Heskett, 1970), at 28 °C, for 48 h. The bacterial suspension was prepared with filtered sterile water, and adjusted to $OD_{550} = 0.5$ (10^9 CFU mL^{-1}) using a spectrophotometer. *X. hortorum* pv. *gardneri* was identified by PCR using the species-specific primer pairs Bs-XgF (5'- TCA GTG CTT AGT TCC TCA TTG TC -3') and Bs-XgR (5'- TGA CCG ATA AAG ACT GCG AAA G-3'), which amplify a 154-bp amplicon (Koenraadt *et al.*, 2009).

In the greenhouse, tomato plants of cv. Santa Cruz Kada were grown in 500-mL pots containing a substrate composed by soil, sand, humus, and vermiculite (4:1:1:1). Thirty-one days after sowing, the plants (three- to four-leaf stage) were sprayed using a hand pump, with ZnO:1Mg, ZnOCl, and ZnOCl:0.1Cu nanocrystals (2.5 mg mL^{-1}), copper hydroxide ($Cu(OH)_2$ contains 0.35 g/L metallic Cu) (2 g L^{-1}), and water (control) until runoff. Three days later, the plant leaves were sprayed with a bacterial suspension (10^9 CFU mL^{-1}). After inoculation, the plants were then sprayed with the products at each 3, 6, 9, or 12-day interval. The plants were kept in a moist chamber for 24 h, before and after inoculation. The temperature inside the greenhouse was measured during carrying out the assay. The nanocrystals with 20 nm approximated size were synthesized at the Laboratory for New Insulating and Semiconductor Materials of UFU's Physics Institute, according to the method described by Silva *et al.* (2018). Nanocrystals used were selected due to the amount of product available in the laboratory, and then the solutions were prepared with filtered sterile water.

During the test, 9, 5, 3, and 3 spraying rounds were performed at 3, 6, 9, and 12-day intervals, respectively. The experiment was in a complete randomized block design in a 5x4 factorial scheme (compounds and control x spraying intervals) with four replications The experimental unit comprised one pot containing two plants. The severity (%) of the disease was analyzed at 3, 6, 9, 12, 15, 18, 21, 24, and 27 days after inoculation, using a diagram scale described by Mello *et al.* (1997).

The area under the disease progress curve (AUDPC) was calculated using the following equation: $AUDPC = \sum\left(\frac{Y_i + Y_{i+1}}{2}\right)\times\left(t_{i+1} - t_i\right)$ in which: Y is disease intensity, t is time, and i is the number of evaluations made over time (Shaner & Finney, 1977). The data obtained were subjected to analysis of variance, and the averages were compared using the Scott-Knott test with

P value of 0.05 of significance using SISVAR software version 5.6 (Ferreira, 2019).

RESULTS AND DISCUSSION

To area under the disease progress curve (AUDPC), for tomato bacterial spot, no significant interaction was observed between the factors (products and spraying intervals). The use of different nanocrystals, and copper showed a significant difference between the water (control), at 3 and 6-day spraying intervals (Table 1). At 3-day spraying interval, the nanocrystals, and copper differed significantly from water (control), showing lower AUDPC for tomato bacterial spot. No difference was observed between ZnO:1Mg nanocrystal and copper, showing similar disease control, both were equally efficient in reducing disease severity for the 3-day spraing interval. The efficay of ZnO:1Mg was higher, compared to ZnOCl and ZnOCl:0.1Cu at 3-day spraying interval, once it significantly reduced the AUDPC for tomato bacterial spot.

No differences were observed in the AUDPC between ZnO:1Mg, ZnOCl, and ZnOCl:0.1Cu nanocrystals, and copper in the 6-day spraying interval, but all differed significantly from the control treatment (water). For the 9 and 12-day spraying intervals, no significant differences were detected between the products and the control treatment (water). AUDPC of tomato bacterial spot at 3, 6, 9, and 12-days spraying intervals showed a lower curve for ZnO:1Mg nanocrystal when compared with water (control), reducing the disease severity, followed by copper.

At 15 days after inoculation (Figure 1A, B, C, D) was observed an increase in the severity progress curve for tomato bacterial spot, especially in plants under the control treatment (water) and at 3, 6, 9, and 12-days spraying intervals. For the 9 (Figure 1C) and 12-day spraying intervals (Figure 1D), disease severity is similar for both treatments using products and the control (water), which indicates that these spraying intervals are not adequate for disease management. In the greenhouse during carrying out the essay, the maximum and minimum temperatures media were 34 and 15 ^{o}C, respectively, temperature adequate for the disease development.

This study showed the potential of the ZnO:1Mg, ZnOCl, and ZnOCl:0.1Cu nanocrystals spraying at 3, and 6-days intervals for the control of tomato bacterial spot. Similar results were observed with the use of essential oils at a 6-day spraying interval, which reduced the severity of tomato bacterial spot, caused by *X. gardneri* (Araújo & Tebaldi, 2019). *X. hortorum* pv. *gardneri* strain from Araguari, MG was used in this study, local with an altitude above 900 m, ideal for specie occurrence, according to Araújo *et al.* (2017). The tomato bacterial spot control observed in this study was probably due to the nanocrystals forme a protective barrier on the plant tissue, before inoculation, making it difficult for the bacteria to penetrate, as related by Fraga *et al.* (2021).

The use of nanocrystals for disease control have been described, such as ZnO nanocrystals doped with Cu, Mn, Ni, Au, and Ag showed *in vitro* bactericidal effect against *P. ananatis*, and ZnO:0.1Cu, and ZnO:0.2Mn nanocrystals at 2.5 mg mL^{-1} reduced the disease severity of maize white spot (Mamede *et al.*, 2022). Fraga *et al.* (2021) observed that ZnO nanocrystals doped with Ag, Au, Cu, Fe, K, Mg, Mn, Mo and Ni inhibited the growth of *X. gardneri in vitro*, ZnO:1K nanocrystal reduced the presence of bacteria in inoculated tomato seeds, and ZnO:0.5Mo, ZnO:1K, and ZnO:1Mg nanocrystals at 2.5 mg mL^{-1} efficiently prevented tomato bacterial spot, in one single application. Also, ZnOCl, ZnOCl:0.1Ag, and ZnOCl:1Cu nanoparticles at 2.5 and 5.0 mg mL^{-1} reduced the tomato bacterial spot, caused by *X. hortorum* pv. *gardneri* in the preventive application

Table 1: Area under the disease progress curve (AUDPC) of tomato bacterial spot at 3, 6, 9, and 12-day spraying intervals using different nanocrystals

	AUDPC			
Products	**3**	**6**	**9**	**12**
ZnO:1Mg	17.62 aA	30.00 aB	41.62 aC	41.62 aC
ZnOCl	34.87 bA	39.37 aA	44.62 aB	51.75 aB
ZnOCl:0.1Cu	31.87 bA	37.12 aA	38.62 aA	46.87 aA
Copper hydroxide	25.12 aA	33.75 aA	44.25 aB	45.37 aB
Water (control treatment)	52.50 cA	48.37 bA	52.87 aA	54.75 aA
CV (%)	19.4			

Averages followed by different small letters in the column and capital letters in the line differ according to the Scott-Knott test, significance at $P = 0.05$.

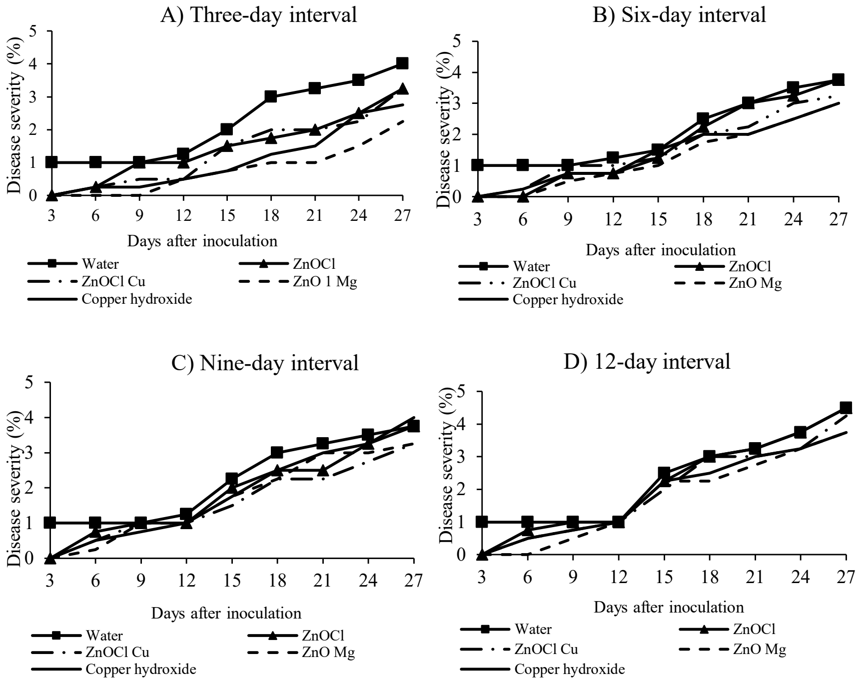

Figure 1: Progress curve for tomato bacterial spot at 3 (A), 6 (B), 9 (C), and 12-day spraying intervals (D), using different nanocrystals..

(Oliveira *et al*., 2023).

The bactericidal action of ZnO nanocrystals may be influenced by their form, concentration and size: the smallest they are, the easiest it is for them to penetrate and damage the bacterial cells (Yamamoto, 2001). When in contact with microorganisms, nanoparticles interact with the cell membrane, producing changes to the respiratory system, cell permeability, and in DNA, leading to programmed cell death (Zhang *et al.*, 2013). The mechanism of action of nanocrystals in microorganisms may be related to a loss in DNA replication capacity and to the inactivation of cellular proteins (Gomaa, 2017), as well as to the increase in reactive oxygen species within the cell, bursting the cells' plasma membrane and thus killing the bacteria (Wang *et al.*, 2014).

The similar performance of ZnO:1Mg nanocrystal, and copper at the 3-day spraying interval indicates that it may be used for the control of tomato bacterial spot, especially considering known cases of *Xanthomonas* strains which are resistant to copper fungicides (Areas *et al.*, 2018), so that, it has a potential to be considered as an alternative to copper bactericides, avoiding the occurrence of copper-resistant strains, and also could be promising due to few formulations registered for disease control in Brazil.

Nanocrystals have potential for control plant diseases. However, there is a need to know the best chemical elements to be used for doping ZnO, dosages and spraying intervals, in order to efficiently manage bacterial plant diseases. Further studies are recommended to evaluate the use of nanocrystals in other *Xanthomonas* species (*X. euvesicatoria* pv. *perforans, X. vesicatoria*, *X. euvesicatoria* pv. *euvesicatoria*) of the complex associated with tomato bacterial spot, as well as other strains of *X. hortorum* pv. *gardneri* to control the disease. In this study, ZnO:1Mg, ZnOCl, and ZnOCl:0.1Cu nanocrystals reduced the severity of tomato bacterial spot when sprayed at 3 and 6-day intervals. The use of nanocrystals showed promising to manage tomato bacterial spot.

CONCLUSIONS

ZnO:1Mg, ZnOCl, and ZnOCl:0.1Cu nanocrystals reduced the disease severity when sprayed at 3- and 6-day intervals.

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