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Simonkolleite nanoparticles for seed treatment and control of tomato bacterial spot caused by *Xanthomonas hortorum* pv. *gardneri*

Nanopartículas de Simonkolleite no tratamento de sementes e controle da mancha bacteriana do tomateiro causada por *Xanthomonas hortorum* pv. *gardneri*

Natália Silva Oliveira[1], Anielle Christine Almeida Silva[2], Nilvanira Donizete Tebaldi[1*]

[1]Universidade Federal de Uberlândia/UFU, Instituto de Ciências Agrárias/ICIAG, Uberlândia, MG, Brasil
[2]Universidade Federal de Alagoas/UFAL, Instituto de Física, Maceió, AL, Brasil
[*]Corresponding author: nilvanira.tebaldi@ufu.br
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ABSTRACT

Bacterial spot caused by *Xanthomonas* spp. (*X. vesicatoria*, *X. euvesicatoria* pv. *euvesicatoria*, *X. euvesicatoria* pv. *perforans*, and *X. hortorum* pv. *gardneri*) may result in significant losses for tomato crops. Simonkolleite nanoparticles (SK-NPs) has been indicated as a novel approach for plant disease control. The objective of this work was to evaluate SK-NPs (ZnOCl, ZnOCl:Ag, and ZnOCl:Cu at different concentrations) for the *in vitro* inhibition of *X. hortorum* pv. *gardneri*, determining the time of exposure of the products for the bacterial death; the reduction of bacteria recovery on inoculated seeds, and their efficacy to reduce bacterial spot severity in plant. The growth inhibition was evaluated by inhibition zone in culture medium plates, when the diameter of the inhibition zone was measured. The period of exposure of the products to the bacterial suspension tested were 1, 2, 3, 4, or 5 h. Tomato inoculated seeds were treated with SK-NPs. In plant, the preventive and curative effects were evaluated by applying the products two days before or after inoculation, respectively. Disease severity was evaluated and the area under the disease progress curve (AUDPC) was calculated. ZnOCl:Ag, ZnOCl:Cu, and ZnOCl inhibited bacterial growth, and the 5 h exposure time was necessary to reduce bacterial growth. ZnOCl:Ag, and ZnOCl:Cu reduced the bacteria presence in the seeds, and did not affect the seed germination. Both products reduced the AUDPC in the preventive application. The use of SK-NPs ZnOCl:Ag and ZnOCl:Cu showed to be promising to manage tomato bacterial spot.

Index terms: Disease control; nanocrystal; severity; *Solanum lycopersicum*.

RESUMO

A mancha bacteriana causada por *Xanthomonas* spp. (*X. vesicatoria*, *X. euvesicatoria* pv. *euvesicatoria*, *X. euvesicatoria* pv. *perforans*, and *X. hortorum* pv. *gardneri*) pode provocar perdas significativas na produção do tomateiro. As nanopartículas de Simonkolleite (NPs-SK) tem sido indicada como uma inovação para o controle de doenças de plantas. O objetivo do trabalho foi avaliar o uso de NPs-SK (ZnOCl, ZnOCl:Ag e ZnOCl:Cu em diferentes concentrações) na inibição do crescimento de *X. hortorum* pv. *gardneri in vitro*, determinar o tempo de exposição dos produtos para causar a morte bacteriana, a redução da bactéria em sementes inoculadas e sua eficácia na redução da severidade da mancha bacteriana em plantas. A inibição do crescimento foi avaliada pela zona de inibição em meio de cultura, quando o diâmetro da zona de inibição foi medido. O período de exposição dos produtos à suspensão bacteriana foi testado por 1, 2, 3, 4 e 5 h. As sementes de tomate inoculadas foram tratadas com as NPs-SK. Em plantas, o efeito da aplicação preventiva e curativa dos produtos foram avaliadas dois dias antes e após a inoculação, respectivamente. A severidade da doença foi avaliada e calculada a área abaixo da curva de progresso da doença (AACPD). ZnOCl:Ag, ZnOCl:Cu e ZnOCl inibiram o crescimento bacteriano e o tempo de exposição de 5 h foi necessário para reduzir a população bacteriana. ZnOCl:Ag e ZnOCl:Cu reduziram a presença da bactéria nas sementes, não afetando a germinação. Os dois produtos reduziram a AACPD em aplicação preventiva. O uso das NPs-SK ZnOCl:Ag e ZnOCl:Cu mostraram promissores para o manejo da mancha bacteriana do tomateiro.

Termos para indexação: Controle da doença; nanocristal; severidade; *Solanum lycopersicum*.

INTRODUCTION

Bacterial spot in tomato plants is caused by four species of *Xanthomonas,* namely, *X. vesicatoria* (Jones et al., 2004), *X. euvesicatoria* pv. *euvesicatoria*, *X. euvesicatoria* pv. *perforans* (Constantin et al., 2016), and *X. hortorum* pv. *gardneri* (Morinièri et al., 2020), and may affect fruit production and quality. It is particularly destructive under conditions of high humidity 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 temperature (20 ºC), and *X. euvesicatoria* pv. *perforans* is predominant and widespread distributed around the country, while *X. vesicatoria* and *X. euvesicatoria* pv. *euvesicatoria* has low occurrence in the tomato fields (Araújo et al., 2017).

There are few products labelled to control the disease. The scarcity of resistant cultivars, the rapid spread of the pathogen between the crop plants, and the fact that the bacterium is transmissible by seeds make control difficult (Nascimento et al., 2013). Chemical control for tomato bacterial spot has been challenging, and the effectiveness of copper fungicides in controlling bacterial spot may have been compromised by the development of resistant populations (Nascimento et al., 2013; Mirik; Aysan; Cinar, 2007). Furthermore, tomato contaminated seeds can spread the bacteria in the field (Carmo et al., 1996) and seed treatment is a management option. In this context, innovative approaches to control bacterial spot and treatment of tomato seeds should be evaluated, such as the use of nanoparticles.

Nanotechnology has been used in human health, environment, and agriculture, in order to eliminate or delay pathogens development (Guzmán; Dille; Godet, 2009). Nanoparticles are smaller than 100 nm in size and show high surface/volume ratio, which give them high efficacy for interacting with and penetrating microbial membranes, thereby facilitating microbial control and/or causing cell death (Morones et al., 2005). At the intracellular level, the membrane is ruptured due to the increase in reactive oxygen species, which causes microorganism death (Wang et al., 2014). Nanoparticles may also form complexes with biomolecules, and lead to damages or to the inactivation of proteins and the inhibition of the bacterial biofilm (Díez-Pascual, 2018; Kalia; Abd-Elsalam; Kuca, 2020).

Several metal oxides may be used to synthesize nanoparticles, such as ZnO, which has low cost and is less toxic to human cells (Premanathan et al., 2011). In tomato plants, ZnO and MgO nanoparticles, respectively, induced systemic resistance (Elsharkawy et al., 2020) and decreased the severity of bacterial spot (Liao et al., 2019).

Simonkolleite nanoparticles (SK-NPs), also named zinc chloride hydroxide monohydrate [$Zn_5(OH)_8Cl_2.H_2O$], or ZnOCl, is a biocompatible mineral built with a structure involving stacked two-dimensional layers (Li et al., 2019), showing bactericidal action because of oxygen vacancies in their surface, and have inhibited bacterial growth when used in bone regeneration applications (Li et al., 2019). To increase their bactericidal effect, nanoparticles can be doped with different elements. Doping is a process that consists of adding new elements to the nanoparticle's structure and is a form of adjusting the properties of functional oxides by altering their physical and electronic structure and changing their chemical characteristics (Kwon et al., 2016). ZnOCl nanoparticles doped with Ag, and Cu inhibited *Alternaria alternata* growth *in vitro* and reduced the pathogen in wheat seeds (Duarte; Catão; Tebaldi, 2022).

The use of SK-NPs doped with silver (ZnOCl:Ag), and copper (ZnOCl:Cu) to control tomato bacterial spot and treat contaminated seeds has not been reported yet, then this work proposes a new strategy to disease management. The hypothesis is that SK-NPs show greater bactericidal potential when compared to copper-based products, and thus render a more efficient control of the disease. Therefore, the objectives of this work were to evaluate the use of ZnOCl:Ag and ZnOCl:Cu at different concentrations to: i) inhibit *X. hortorum* pv. *gardneri* growth *in vitro;* ii) evaluate the time of exposure of the bacterial suspension to cause cell death; iii) treat inoculated seeds to eliminated the bacteria from them; iv) reduce the tomato bacterial spot severity in plants under controlled conditions.

MATERIAL AND METHODS

The *Xanthomonas hortorum* pv. *gardneri* strain UFU A35 (copper-sensitive), preserved and maintained in the work collection of Laboratório de Bacteriologia Vegetal, Universidade Federal de Uberlândia (UFU), Minas Gerais, Brazil, was grown using medium 523 (Kado; Heskett, 1970) at 28 ºC. After 48 h, the bacterial suspension was prepared using sterile, filtered water, and adjusted to OD_{550} = 0.5 [10^9 Colony Forming Units (CFU) mL^{-1}] using a spectrophotometer.

Inhibition of *in vitro* bacterial growth

The experiment design was completely randomized with three replications, in a 9 x 4 +2 factorial scheme, with 9 SK-NPs, 4 serial dilutions [non-diluted (ND), and 10^{-1} to 10^{-3}], plus 2 additional control treatments (cephalexin and NaCl).

The following SK-NPs were tested for their bacterial activity ZnOCl, and ZnOCl doped with silver (Ag) at ZnOCl:0.1Ag, ZnOCl:1.0Ag, ZnOCl:5.0Ag, ZnOCl:10.0Ag, and ZnOCl doped with copper (Cu) at ZnOCl:0.1Cu, ZnOCl:1.0Cu, ZnOCl:5.0Cu, ZnOCl:10.0Cu. The SK-NPs were synthesized at the Laboratório de Novos Materiais Isolantes e Semicondutores, Instituto de Física, UFU, according to the

method described by Silva et al. (2018). The approximate size of the SK-NPs was 20 nm. Suspensions for each SK-NP were prepared using filtered, sterile water at 10 mg mL^{-1} concentrations, and then serially diluted (10^{-1} to 10^{-3}).

A base layer of 2% agar-water medium and semi-solid nutrient medium (0.8%) supplemented with 10% bacterial suspension was added to Petri dishes (8 cm). Six sterile filter paper disks (6 mm) were placed onto the medium and soaked with 10 µL of each SK-NP solution at different concentrations, 500 µg mL^{-1} of cephalexin (positive control), and 0.45% NaCl (negative control). Each of the treatments had three replicates (Petri dishes). After incubation at 28 ºC for 48 h, the diameters (mm) of the inhibition zones were measured using a slide gauge. The data obtained were subjected to analysis of variance and the means were compared by the Tukey, and Dunnet tests, with a *P* value of 0.05 using R v 4.0.2 software (R Core Team, 2020). ZnOCl:0.1Ag, ZnOCl:1.0 Cu, and ZnOCl were select for subsequent treatments due to the cost of synthesis and in function of the inhibition zone.

Time exposure to cause *in vitro* cell death

A 100 µL of bacterial suspension (10^9 CFU mL^{-1}) and 900 µL of SK-NP (ZnOCl:0.1Ag, ZnOCl:1.0 Cu, and ZnOCl) solutions at 0.1 mg mL^{-1} were added to sterile microtubes (1.5 mL), mixed, and left to stand for 0, 1, 2, 3, 4, or 5 h at 28 °C. The treated bacterial suspensions were then serially diluted (10^{-2} to 10^{-7}), and 100 µL of each dilution was grown on medium 523 at 28 ºC for 72 h. Colony-forming units mL^{-1} were quantified, and the amount of dead bacterial cells was assessed (Fraga et al., 2021).

Bacteria detection in treated inoculated seeds

The experimental design was completely randomized with four replications, in a 4 x 2 factorial scheme, with 4 treatments (3 SK-NPs and control), and 2 conditions seeds (inoculated or uninoculated).

Commercial seeds of the tomato cultivar Santa Cruz Kada with no prior treatment were disinfested using alcohol at 50% for 30 s, and 1% NaClO for 3 min. The seeds were subsequently rinsed using sterile water and dried on sterile filter paper under laminar flow conditions.

To inoculate the seeds, the bacterium was grown on medium 523 at 28 ºC for 48 h. Then, 5 mL of 0.45% NaCl were added to the bacterial growth and homogenized using a Drigalski loop. After that, 25 seeds were placed in each plate, for 10 min. The seeds were then removed and set to dry on filter paper under laminar flow conditions. A total of 1,200 seeds were inoculated. The inoculated or uninoculated seeds were treated for 10 min with the ZnOCl, ZnOCl:0.1Ag, and ZnOCl:1.0 Cu solutions at 5 mg mL^{-1} or the control treatment (0.45% NaCl), and then dried on filter paper.

To detect the bacteria in the seeds, 100 seeds from each treatment were divided into four replicates of 25 seeds, which were placed in Petri dishes containing culture medium 523 supplemented with cycloheximide (0.5 mL mL^{-1}) to avoid saprophytic growth, and incubated at 28 ºC for 48 h. Thereafter, the percentage of contaminated seeds was assessed.

The seed germination test was performed according to the Seed Analysis Rules (Brasil, 2009) using 200 seeds for each treatment, with four replicates of 50 seeds. The seeds were placed on two sheets of blotting paper in gerbox-type boxes, and the paper was soaked with a water amount equivalent to 2.5 times the paper's dry mass. The seeds were then placed in a germinator chamber at 20–30 °C and under a photoperiod of 16 h light/8 h dark. The evaluations consisted of counting the number of normal seedlings that developed from the germinated seeds. The first and final counts to determine vigor and germination were performed on days five and fourteen, respectively. The results were expressed in germination percentage. The data obtained were subjected to analysis of variance, and the means were compared by the Tukey test, with a *P* value of 0.05 using the Sisvar software (Ferreira, 2019).

Severity of bacterial spot in preventive and curative applications

The experimental design was randomized complete block with four replications, in a 2 x 8 factorial scheme, with 2 applications (preventive and curative), 8 treatments (3 SK-NPs × 2 concentrations – 2.5 or 5.0 mg mL^{-1} –, copper, water). Each experimental unit comprised one pot containing 2 plants.

Plants of tomato cv. Santa Cruz Kada were grown in a greenhouse, in 500-mL pots containing a mixture of soil, sand, and vermiculite (3:1:1). For the evaluation of the preventive and curative effect of the SK-NPs application, the plants, at 3-4 true-leaves stage (30 days after sowing), were treated two days before or after the inoculation, respectively. The plants were sprayed until runoff with ZnOCl:0.1Ag, ZnOCl:1Cu, and ZnOCl solutions, at two concentrations (2.5 or 5.0 mg mL^{-1}), copper hydroxide (2 mg mL^{-1}), and water. The plants' leaves were sprayed until runoff with a bacterial suspension (10^8 CFU mL^{-1}). The plants were kept in a moist chamber for 24 h before and after inoculation, at 30 ºC. Copper hydroxide contains 35% metallic Cu in the form of copper hydroxide [$Cu(OH)_2$].

Copper hydroxide at 2 mg mL^{-1} contains approximately 0.7 mg mL^{-1} of Cu.

Disease severity was assessed at 3, 6, 9, 12, and 15 days after inoculation using a diagram scale (Mello; Takatsu; Lopes, 1997). The area under the disease progress curve (AUDPC) was calculated using the following formula: $AUDPC = \sum [(Y_i + Y_{i+1})/2] (t_{i+1} - t_i)$, in which Y is disease intensity, t is time (interval between assessments, in days), and i is the number of assessments made over time (Shaner; Finney, 1977). The data obtained were subjected to an analysis of variance, and the means were compared using the Scott Knott test with a *P* value of 0.05 (Ferreira, 2019).

RESULTS AND DISCUSSION

Inhibition of *in vitro* bacterial growth

ZnOCl SK-NPs, pure or doped with Ag and Cu, at different concentrations of the doping element at 10 mg mL^{-1} (ND) and different dilutions (10^{-1} to 10^{-3}), and cephalexin inhibited *X. hortorum* pv. *gardneri* growth (Table 1). The halos varied from 51 to 2 mm, with the exceptions of ZnOCl:1Ag, ZnOCl:1Cu, and ZnOCl at a 10^{-3} dilution, which did not inhibit bacterial growth. In the controls, inhibition halos were not observed for NaCl, but were present for cephalexin. The strongest inhibition zone was observed under undiluted (10 mg mL^{-1}) differing to the diluted (10^{-1} to 10^{-3}) solutions. The inhibition of the bacterial growth shows that SK-NPs have toxic effects on *X. hortorum* pv. *gardneri,* and also on *Alternaria alternata* (Duarte; Catão; Tebaldi, 2022). The antimicrobial activity of ZnO particles doped with Ag (ZnO:Ag) was observed on *Pantoea ananatis* at 10 mg mL^{-1} (Mamede et al., 2022), and on *Alternaria alternata* at 2.5 and 5 mg mL^{-1} (Duarte; Catão; Tebaldi, 2022), also Cu and Ag nanoparticles were toxic to *Escherichia coli*, *Bacillus subtilis, Staphylococcus aureus,* and *Ralstonia solanacearum* (Ruparelia et al., 2008; Santiago et al., 2019). The bactericidal effect of SK-NPs may be associated with the release of reactive oxygen species, leading to the destruction of the cell's membranes, and causing damages to the bacterial DNA (Wang et al., 2014).

Time exposure to cause *in vitro* cell death

The 5 h exposure time of the bacterial suspension to the ZnOCl, ZnOCl:0.1Ag, and ZnOCl:1Cu solutions at 0.1 mg mL^{-1} was necessary to reduce bacterial growth from 10^8 CFU mL^{-1} to 120, 12, and 8 CFU mL^{-1}, respectively, and was also adequate to cause bacterial death. The 0, 1, 2, 3, and 4 h exposure time was not sufficient to reduce the bacterial population to a countable number.

Table 1: Growth inhibition zone (mm) for *Xanthomonas hortorum* pv. *gardneri* treated with Simonkolleite nanoparticles (SK-NPs) doped with silver (Ag) and copper (Cu) at 10 mg mL^{-1} (non-diluted ND) and serially diluted (10^{-1} to 10^{-3}).

SK-NPs	Dilution			
	ND	10^{-1}	10^{-2}	10^{-3}
ZnOCl:0.1Ag	39.4 abA^{+}	21.8 bB*$^{+}$	10.2 bC*$^{+}$	3.1 aD*
ZnOCl:1.0Ag	20.4 cA*$^{+}$	9.9 cB*$^{+}$	6.5 bcB*$^{+}$	0.0 aC*
ZnOCl:5.0Ag	45.5 abA^{+}	38.2 aB^{+}	10.7 bC*$^{+}$	4.0 aD*
ZnOCl:10.0Ag	51.0 aA*$^{+}$	28.4 bB^{+}	17.4 aC*$^{+}$	5.0 aD*
ZnOCl:0.1Cu	31.5 bA^{+}	15.6 cB*$^{+}$	9.8 bB*$^{+}$	4.0 aC*
ZnOCl:1.0Cu	41.7 abA^{+}	21.1 bcB*$^{+}$	10.4 bC*$^{+}$	0.0 aD*
ZnOCl:5.0Cu	28.2 bA^{+}	12.3 cB*$^{+}$	9.9 bB*$^{+}$	2.0 aC*
ZnOCl:10.0Cu	42.2 abA^{+}	9.4 cB*$^{+}$	6.0 bcB*$^{+}$	2.4 aC*
ZnOCl	18.0 cA*$^{+}$	8.3 cB*$^{+}$	6.5 bcB*$^{+}$	0.0 aC*
Cephalexin	33.5			
NaCl	0.0			
CV (%)	23.58			

Means followed by different lowercase letters in the column and uppercase letters in the row are significantly different, as determined using the Tukey test (*P* = 0.05). Cephalexin* and NaCl^{+}: means compared to the control treatments are significant according to the Dunnett test (*P* = 0.05).

The longer contact time (5 h) between the SK-NPs solution and bacterial cells, the lower colonies number of *X. hortorum* pv. *gardneri* was counted, and the toxicity depends on the exposure time, as well as observed in the same bacteria using ZnO:1K nanoparticle (Fraga et al., 2021). Bacteria intoxication by Ag nanoparticles is a process caused by the release of Ag^{+} ions, which interact with the thiol group of proteins and, therefore, damage the respiratory chain by reducing ATP production, or interact with DNA, and impair regulation gene by alteration of DNA replication (Marini et al., 2007), and this antimicrobial capacity is strengthened in the form of nanoparticles (Panacek et al., 2006). Nanoparticles enter the cell membrane easily, but a time is necessary for the penetration into the bacteria cell, and the toxicity depends on the exposure time, between 2 and 24 h (Grigore et al., 2016).

Bacteria detection in treated inoculated seeds

X. hortorum pv. *gardneri* was detected in 98% of the untreated inoculated seeds (control) (Table 2), which significantly differed from the inoculated seeds that had been treated with ZnOCL:0.1Ag (81%), ZnOCl:1.0Cu (85%), and ZnOCl (87%). No bacteria were detected in uninoculated seeds (data not shown here).

The germination percentage did not differ significantly among inoculated or uninoculated tomato seeds treated with SK-NPs or control, which ranged from 94% to 97% (Table 2), and no significant difference were observed between inoculated and uninoculated seeds. Thus, the SK-NPs did not interfere in the germination of tomato seeds, and also in the wheat seeds (Duarte; Catão; Tebaldi, 2022).

Tomato seeds contaminated by bacteria may set off several epidemics, both in nurseries and on the field, even at low levels of contamination. Sweet pepper seeds showing 0.01% contamination by *X. vesicatoria* rendered 100% contamination of seedlings in a nursery 30 days after sowing (Carmo et al., 1996). Seed treatment to control bacterial diseases is a management option to prevent stand reduction and improve seedling performance, because the plants are healthier at the beginning of their lifecycle and show better production conditions (Carmo et al., 1996).

The SK-NPs significantly reduced the number of bacteria detected in the tomato seeds and did not affect their germination. They also had no detectable phytotoxic effects on the seeds. Accordingly, SK-NPs can be an alternative to be used on treat tomato seeds and control *X. hortorum* pv. *gardneri,* avoiding bacteria dissemination, and therefore be a new strategy for treatment of tomato seeds.

Severity of bacterial spot in preventive and curative application

The AUDPC for tomato bacterial spot was reduced using different SK-NPs, and also showed significant difference between the applications before and after inoculation (Table 3). The application before inoculation significantly reduced AUDPC when compared with the application after inoculation. The application before inoculation (146.4) was also efficient to reduce the AUDPC and to control tomato bacterial spot, while the application after inoculation (182.5) was not efficient to control the disease. In the application before inoculation, ZnOCl:0.1 Ag at 2.5 mg mL^{-1} (114.3), and ZnOCl at 5 mg mL^{-1} (132.2) concentrations significantly reduced AUDPC; for the other SK-NPs at different concentrations, the control treatment showed similar results to copper hydroxide (144.1) when compared with plants sprayed with water (190.5). In the application after inoculation, ZnOCl:0.1Ag (154.5), ZnOCl:1.0Cu (154.5), and ZnOCl (165.7) at 5.0 mg mL^{-1} significantly reduced the AUPDC, as did metallic copper hydroxide (185.5), when compared with water (206.8). Applications of SK-NPs before inoculation at 2.5 and 5 mg mL^{-1}, and after inoculation at 5 mg mL^{-1} reduced the AUDPC.

The application of SK-NPs before inoculation rendered less severe disease scenarios when compared to the after inoculation, probably due to the formation of a protective barrier – by the SK-NP as well as the copper hydroxide – on the plants tissue before inoculation, thus making it difficult for the bacteria to penetrate it. In the application after inoculation, there was no formation of such barrier. In addition, the ZnO nanoparticles applied in the preventive application could induce a plants defense mechanism, which was observed in a study with bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (Elsharkawy et al., 2020). Tomato plants treated with ZnO nanoparticles showed higher self-defense enzyme activity and consequently developed systemic resistance (Elsharkawy et al., 2020), protecting the plants against the pathogen.

Antimicrobial activity of ZnO and ZnO-nCuSi nanoparticles was comparable to or greater than that of commercial copper formulations to control citrus canker caused by *X. citri* subsp. *citri*, which was likely due to the translaminar movement of the nanoparticles (Graham et al., 2016; Young et al., 2018). Copper-based commercial products show low efficiency to control bacterial diseases and also favor the development of resistant populations (Mirik; Aysan; Cinar, 2007). The use of Cu as doping element in nanoparticles may be an innovative alternative to control tomato bacterial spot at lower costs when compared to Ag nanoparticles.

Table 2: Detection of *Xanthomonas hortorum* pv. *gardneri* in inoculated seeds and germination percentage of inoculated or uninoculated tomato seeds treated with Simonkolleite nanoparticles.

	Detection (%)	Germination (%)^{ns}		
	Inoculated	Inoculated	Uninoculated	Mean
ZnOCl:0.1Ag	81 a	95	97	96 a
ZnOCl:1.0Cu	85 a	97	96	97 a
ZnOCl	87 a	96	94	95 a
Control	98 b	97	96	97 a
Mean	88	96 A	96 A	
CV (%)	52	16		

Means followed by different lowercase letters in the column and uppercase letters in the row are significantly different, as determined by the Tukey test (P = 0.05), ns = not significant (P. value > 0.05).

Table 3: Area under the disease progress curve (AUDPC) for tomato bacterial spot using different Simonkolleite nanoparticles in applications before and after inoculation.

Products	AUDPC	
	Before inoculation	After inoculation
ZnOCl:0.1Ag [2.5]	114.3 aA	191.6 cB
ZnOCl:0.1Ag [5.0]	152.4 bA	154.5 aA
ZnOCl:1.0Cu [2.5]	152.5 bA	195.7 cB
ZnOCl:1.0Cu [5.0]	143.2 bA	154.5 aB
ZnOCl [2.5]	142.8 bA	206.1 cB
ZnOCl [5.0]	132.2 aA	165.7 aB
Copper hydroxide	144.1 bA	185.5 bB
Water (control)	190.5 cA	206.8 cA
Application mean	146.4 A	182.5 B
CV (%)	10.9	

Means followed by different lowercase letters in the column and uppercase letters in the row are significantly different, according to the Scott Knott test (P = 0.05).

The effectiveness of ZnO-nanoparticle formulations to control phytopathogens is due to its versatile antimicrobial action, which encompasses photo-oxidation and produces reactive oxygen species, destabilization of cell membrane, organelles and other cellular macromolecules, and toxicity, due to the release of zinc ions (Kalia; Abd-Elsalam; Kuca, 2020). Nanoscale formulations of TiO_2 can damage the outer membrane, penetrating into the bacterial cytoplasm and reduced tomato bacteria spot severity caused by *X. euvesicatoria* pv. *perforans* (Paret et al., 2013).

Simonkolleite nanoparticles at 2.5 or 5.0 mg mL^{-1} concentrations did not show signs of phytotoxicity to tomato. Applications of ZnOCl:Ag and ZnOCl before inoculation reduced AUDPC, and showed potential for use in the management of tomato bacterial spot. They may also be good chemical alternatives to copper bactericides. ZnOCl:0.1Ag at 2.5 mg mL^{-1} concentration decreased the disease severity compared with the 5 mg mL^{-1} before inoculation; 2.5 mg mL^{-1} was more efficient than 5 mg mL^{-1}; due to low concentration, less aggregation of the product occurs and, consequently, an increase of the exposed reactive surface area of the nanoparticle with the microorganism, improving the bactericide action.

Although the seeds treatment with SK-NPs was not enough to reduce the bacterial population to a low level, this could be related to the high concentration of the bacteria present in the seeds at inoculation time. However, SK-NPs could be recommended for the treatment of tomato seeds, and also for the management of bacterial spot in preventive applications, and are therefore a promising alternative to control the pathogen. This is the apparently the first report of the use of ZnOCl nanoparticles doped with Ag and Cu to control tomato bacterial spot, and to treat tomato seeds. The data suggest that the complementary experiments should be carried out using other doses of the nanoparticles to assess their efficacy in controlling the disease. Furthermore, other studies are needed to assess whether SK-NPs will be effective for other *Xanthomonas* species of the complex that causes tomato bacterial spot, as well as in others strains of *X. hortorum* pv. *gardneri*.

CONCLUSIONS

ZnOCl:0.1Ag, ZnOCl:1Cu, and ZnOCl nanoparticles inhibited *X. hortorum* pv. *gardneri* growth,

5 h exposure time was necessary to reduce bacterial growth, the presence of the bacteria on tomato seeds was reduced, and the seed germination was not affected. Tomato bacterial spot severity was reduced with the application of ZnOCl:0.1Ag at 2.5 mg mL^{-1}, and ZnOCl at 5 mg mL^{-1} before inoculation, and ZnOCl:0.1Ag, ZnOCl:1.0Cu and ZnOCl at 5 mg mL^{-1} after inoculation. The application of SK-NPs in preventive application reduced the disease severity.

AUTHOR CONTRIBUTION

Conceptual idea: Tebaldi, N.D.; Methodology design: Tebaldi, N.D.; Oliveira, N.S.; Data collection: Oliveira, N.S.; Data analysis and interpretation: Oliveira, N.S.; Tebaldi, N.D.; Writing and editing: Oliveira, N.S.; Tebaldi, N.D.

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