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Dickeya zeae, Pantoea ananatis, and *Xanthomonas vasicola* pv. *vasculorum*: Control with the use of nanoparticles

Dickeya zeae, *Pantoea ananatis* e *Xanthomonas vasicola* pv. *vasculorum*: Controle com o uso de nanopartículas

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ABSTRACT

Metal oxide nanoparticles (NPs) possess antibacterial activities and can be used for the control of phytopathogenic bacteria. The objective of this work was to evaluate the antibacterial activity of pure and doped NPs against *Dickeya zeae*, *Pantoea ananatis,* and *Xanthomonas vasicola* pv. *vasculorum in vitro,* and to assess the efficacy of preventive and curative application of zinc oxide (ZnO), nickel oxide (NiO), and silver-doped zinc oxide (ZnO:Ag) NPs for the control of white spot (WS) and bacterial leaf streak (BLS) *in vivo*. Bacterial growth inhibition was first evaluated by measuring the diameter of the inhibition zone formed in Petri dishes. Subsequently, the severity of WS and BLS diseases was evaluated in a greenhouse calculating the area under the disease progress curve. The *in vitro* antibacterial activity was not influenced by the increase in the concentration of doping elements for most NPs. ZnO NPs doped with Ag, K, and Mo; ZnOCl doped with Ag, and pure NPs (Ag_2O, CuO, and NiO) showed antibacterial activity against *D. zeae*, *P. ananatis,* and *X. vasicola* pv. *vasculorum* with relatively similar inhibition zones at different concentrations. Commercial copper showed antibacterial activity only against *D. zeae*. NiO NPs in preventive and curative applications reduced WS and BLS severities, whereas commercial copper application increased WS severity and reduced BLS severity. The use of NPs has promising applications and further evaluation of their formulation, application form, and timing is necessary for new strategies to control the activity of phytopathogenic bacteria.

Index terms: Bacterial leaf streak; bacterial wilt; severity; white spot; *Zea mays*.

RESUMO

Nanopartículas de óxido metálico (NPs) possuem atividade antibacteriana e podem ser utilizadas para o controle de bactérias fitopatogênicas. O objetivo deste trabalho foi avaliar a atividade antibacteriana de NPs puras e dopadas contra *Dickeya zeae*, *Pantoea ananatis* e *Xanthomonas vasicola* pv. *vasculorum in vitro*, e avaliar a eficácia da aplicação preventiva e curativa de NPs de óxido de zinco (ZnO), óxido de níquel (NiO) e óxido de zinco dopado com prata (ZnO:Ag) para o controle da mancha branca (WS) e estria bacteriana (BLS) *in vivo*. A inibição do crescimento bacteriano foi primeiramente avaliada medindo o diâmetro da zona de inibição formada em placas de Petri e a severidade das doenças WS e BLS foi avaliada em casa de vegetação, calculando a área abaixo da curva de progresso da doença. A atividade antibacteriana *in vitro* não foi influenciada pelo aumento na concentração dos elementos dopantes para a maioria das NPs. NPs de ZnO dopadas com Ag, K e Mo; ZnOCl dopada com Ag e NPs puras (Ag_2O, CuO e NiO) apresentaram atividade antibacteriana contra *D. zeae*, *P. ananatis* e *X. vasicola* pv. *vasculorum* com halos de inibição relativamente semelhantes em diferentes concentrações. O cobre comercial apresentou atividade antibacteriana apenas para *D. zeae*. NPs de NiO em aplicações preventivas e curativas reduziram a severidade de WS e BLS, enquanto a aplicação de cobre comercial aumentou a severidade de WS e reduziu a severidade de BLS. O uso de NPs tem aplicações promissoras na cultura do milho e estudos futuros avaliando sua formulação, forma de aplicação e época são necessários para novas estratégias de controle de bactérias fitopatogênicas.

Termos de indexação: Eestria bacteriana; podridão bacteriana; severidade; mancha branca; *Zea mays*.

Introduction

Brazil is the third largest producer of maize (116.0 million tons) in the world, behind China (272.6 million tons) and the USA (382.9 million tons) (Fiesp, 2022). Maize production is predicted to increase to approximately 125.8 million tons (6000 kg ha^{-1}) in the 2022–23 harvest season in Brazil (Conab, 2022). However, it is affected by several bacterial diseases, including white spot (WS), caused by *Pantoea ananatis* (Paccola-Meirelles et al., 2001); bacterial leaf streak (BLS), caused by *Xanthomonas vasicola* pv. *vasculorum* (Lang et al., 2017); and bacterial wilt, caused by *Dickeya zeae* (Sin: *Erwinia chrysanthemi* pv. *zeae*) (Samson et al., 2005).

WS occurs in all maize-producing regions and is considered endemic to Brazil (Escanferla et al., 2018). Considering the leaf

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area infected, disease severity of 10%–20% causes up to 60% yield losses owing to the decreased photosynthetic area (Godoy, Amorim, & Bergamin Filho, 2001; Casela, Ferreira, & Pinto, 2006; Pinto, 1999). Recently, BLS of maize was confirmed in Brazil (Leite et al., 2019; Sagata et al., 2018) and in the USA with incidence rates of more than 90% and infected leaf area over 50% (Arias et al., 2020; Ortiz-Castro et al., 2020; Leite Jr et al., 2018; Plazas et al., 2018; Korus et al., 2017; Lang et al., 2017). Additionally, the bacterial wilt identified in Brazil by Reifschneider and Lopes (1982) can infect one or several internodes of corn above the soil surface (Kumar et al., 2016), with symptoms such as soft rot and darkening of the infected tissues resulting in severe grain losses (Kumar et al., 2015).

Several reviews provide insight into the latest developments in nanoparticle research (Coelho et al., 2024; Lade et al., 2019; Vurro et al., 2019; Kitherian 2016; Fernandes et al., 2014; Gogos, Knauer, & Bucheli, 2012). Recently, nanoparticles (NPs) have attracted considerable attention as an innovative alternative for the control of bacterial diseases owing to their bactericidal activity (Oliveira et al., 2023; Duarte, Catão, & Tebaldi, 2022; Mamede et al., 2022; Fraga et al., 2021; Silva et al., 2021; Qi et al., 2020).

NPs are nanoscale substances (1–100 nm) that exhibit a high surface-to-volume ratio (Kah & Hofmann, 2014), with physical (Silva et al., 2017) and biological (Reis et al., 2015) activities that facilitate their interaction with the cell membrane. Particularly, metal oxide NPs are widely used in the fields of physics, chemistry, and biology (Kaviya et al., 2011). Notably, their antibacterial activity can occur due to destabilization, degradation, or dissolution of the bacterial cell membrane, allowing NPs to interact with internal cell compounds. The release of toxic ions in the cell membrane physically blocks cellular transport channels (Hou et al., 2018). Moreover, as higher the production of reactive oxygen species (ROS) is, higher the efficacy of the NPs (Lin et al., 2017; Ning et al., 2017).

A previous study confirmed the antibacterial activity of ZnO:Ag NPs against *P. ananatis in vitro* (Mamede et al., 2022). Additionally, potassium-doped zinc oxide (ZnO:K), magnesium-doped zinc oxide (ZnO:Mg), and molybdenum-doped zinc oxide (ZnO:Mo) NPs reduced the population of *Xanthomonas gardneri* in tomato seeds (Fraga et al., 2021). NPs must be biocompatible and specific (Sousa et al., 2014) to avoid the development of resistant pathogenic bacteria (Neves et al., 2021; Pavão et al., 2021). Considering the need for alternative products for managing bacterial diseases, this study aimed to assess the *in vitro* antibacterial activity of pure and doped NPs against *D. zeae*, *P. ananatis*, and *X. vasicola* pv. *vasculorum,* and to evaluate the preventive and curative applications of ZnO, NiO, and ZnO:Ag NPs for the control of WS and BLS in maize.

Material and Methods

The experiment was conducted at the Laboratório de Bacteriologia Vegetal (LABAC) and in a greenhouse of the Instituto de Ciências Agrárias, Universidade Federal de Uberlândia (UFU), Minas Gerais, Brazil.

Isolates of *D. zeae* (UFU M42-5), *P. ananatis* (UFU A18), and *X. vasicola* pv. *vasculorum* (UFU J29) were grown on medium 523 (Kado & Heskett, 1970) for 24 – 48 h at 28 °C. Afterward, aqueous suspensions of bacteria were prepared for each isolate and adjusted to $OD_{550} = 0.5$ (approximately 10^9 CFU mL^{-1}) using a spectrophotometer.

Pure and doped NPs were synthesized in the Laboratório de Novos Materias Isolantes e Semicondutores, Instituto de Física, UFU, according to Silva et al. (2018). The same batch of NPs was used in both trials (*in vitro* and in the greenhouse) and the NPs suspensions were prepared using sterilized tap water (Table 1). For the *in vitro* assay, stock solutions (10 mg mL^{-1}) were serially diluted (10^{-1} to 10^{-2}). For the assays at the greenhouse, a dose of 2 mg mL^{-1} was used.

Table 1: Nanoparticles and their respective concentrations.

Doped NPs	Concentration (%)			
ZnO Ag	0.7	1	9	11
ZnO Cu	1	4	8	
ZnO Fe	0.5	1	5	
ZnO K	0.5	1	5	10
ZnO:Mg	0.5	1		
ZnO:Mn	0.2	0.4	0.8	
ZnO:Mo	0.5	1	5	10
ZnO:Ni	0.3	1	3	
ZnOCl:Ag	0.1	1	5	10
ZnOCl:Cu	0.1	1	5	10

Pure NPs	ZnO	AgO	CuO	FeO	MnO	NiO	ZnOCl

ZnO:Ag: silver-doped zinc oxide; ZnO:Cu: copper-doped zinc oxide; ZnO:Fe: iron-doped zinc oxide; ZnO:K: potassium-doped zinc oxide; ZnO:Mg: magnesium-doped zinc oxide; ZnO:Mn: manganese-doped zinc oxide; ZnO:Mo: molybdenum-doped zinc oxide; ZnO:Ni: nickel-doped zinc oxide; ZnOCl:Ag: silver-doped simonkolleite; ZnOCl:Cu: copper-doped simonkolleite; ZnO: zinc oxide; AgO: silver oxide; CuO: copper oxide; FeO: iron oxide; MnO: manganese oxide; NiO: nickel oxide; ZnOCl: simonkolleite.

Inhibition of bacterial growth *in vitro*

The experimental design was completely randomized, with NPs in their different doping concentrations and two additional ones (commercial copper and pure NPs) with three replications. The bactericidal activities of pure NPs (ZnO, ZnOCl, Ag_2O, CuO, FeO, MnO, and NiO), doped NPs (ZnO:Ag, ZnO:Cu,

ZnO:Fe, ZnO:K, ZnO:Mg, ZnO:Mn, ZnO:Mo, and ZnO:Ni), and simonkolleite NPs (ZnOCl:Ag and ZnOCl:Cu) were evaluated against *D. zeae* and *X. vasicola pv. vasculorum*. Since the bactericidal activities of the other NPs have been previously evaluated (Mamede et al., 2022), only the bactericidal activities of ZnO:K, ZnO:Mo, ZnOCl, ZnOCl:Ag, and ZnOCl:Cu NPs were assessed against *P. ananatis*. Moreover, the antibacterial activity of commercial copper - 35% metallic Cu in the form of copper hydroxide ($Cu(OH)_2$) (2 mg mL^{-1}) for each pathogen was also assessed.

To evaluate the bacterial growth inhibitory effects of the NPs, a base layer of 2% water-agar medium and 0.8% semi-solid nutrient medium supplemented with 10% bacterial suspension was added to Petri dishes (Romeiro, 2001). Thereafter, five sterilized filter paper disks (6 mm diameter) were placed in the center of the medium and soaked with 10 µL of each NP solution at the various dilution rates, water (negative control), and cephalexin or streptomycin (positive control). The Petri dishes were incubated at 28 °C for 72 h, and the diameters of the growth inhibition zones formed around the disks were measured in centimeters (cm) using a slide gauge (Mamede et al., 2022).

Preventive and curative applications of nanoparticles to control white spot and bacterial leaf streak in maize

The experiment was arranged in a 2 × 7 factorial design comprising two applications (preventive and curative) and seven treatments (NPs: ZnO, NiO, ZnO:0.7Ag, ZnO:1.0Ag, and ZnO:9.0Ag; commercial copper; and water) in four replicates. Each experimental unit comprised two maize plants (hybrid P3551 PW) grown in 500 mL pots containing soil, sand, and vermiculite (3:1:1).

Isolates of *P. ananatis* and *X. vasicola* pv. *vasculorum* were grown on culture medium 523 (Kado & Heskett, 1970) at 28 °C. After 48 h, bacterial suspension was prepared in sterile filtered water and calibrated to OD_{550} = 0.1 (1 × 10^8 CFU mL^{-1} for *X. vasicola* and 1 × 10^{10} CFU mL^{-1} for *P. ananatis*) with a spectrophotometer using the count plate method. Subsequently, *P. ananatis* suspension was diluted to a concentration of 1 × 10^8 CFU mL^{-1} also using the same method.

After 15 days of sowing (3- to 4- leaf stage of maize), leaves were sprayed until runoff with solutions of ZnO, ZnO:0.7Ag, ZnO:1.0Ag, ZnO:9.0Ag, and NiO NPs (2 mg mL^{-1}); commercial copper (2 mg mL^{-1}), or water (control). Three days later, leaves were inoculated with bacterial suspensions (1 × 10^8 CFU mL^{-1}) in the preventive application. To evaluate the curative effects, plants were first inoculated with the bacterial suspensions (*P. ananatis* and *X. vasicola* pv. *vasculorum* individually and a mixture of both suspensions), and 3 days later sprayed until runoff with the NP solutions, copper, and water. Plants were kept in an improvised humid chamber for 24 h before and after inoculation.

The severity of WS and BLS were evaluated at 3, 6, 9, and 12 days after inoculation using a diagrammatic scale proposed by Malagi et al. (2011) and Braga et al. (2020), respectively. The area under the disease progress curve (AUDPC) was calculated using the following formula: $AUDPC = \Sigma ((Y_i + Y_{i+1})/2) (t_{i+1} - t_i)$, where Y is the disease intensity; t is time (interval between evaluations, in days); and i is the number of evaluations over time (Shaner & Finney, 1977).

Data were subjected to analysis of variance and means were compared using Tukey's test, Scott–Knott, and Dunnett's test at a 5% probability. All analyses were performed using R v 4.0.2 (R Core Team, 2020).

Results and Discussion

Inhibition of bacterial growth *in vitro*

Pure ZnO and ZnOCl NPs, as well as NPs dilutions of 10^{-1} and 10^{-2} (data not shown), did not significantly inhibit the growth of *D. zeae, P. anantis,* and *X. vasicola* pv.*vasculorum* (Tables 2, 3, and 4).

Table 2: Growth inhibition zones (cm) of different nanoparticles against *Dickeya zeae* at 10 mg mL^{-1}.

Doped NPs	Concentrations of doping agents				Pure NPs
ZnO:Ag	0.7	1.0	9.0	11.0	*AgO
	0.00 B*+	0.70 A+	0.80 A+	0.00 B*+	0.80
ZnOCl:Ag	0.1	1.0	5.0	10.0	CuO
	0.00 B*+	0.79 A+	0.00 B*+	0.80 A+	0.80
ZnO:K	0.5	1.0	5.0	10.0	NiO
	0.95 A	0.85 A+	0.00 B+	0.00 B+	0.80
ZnO:Mg	0.5	1.0			
	0.00 B+	0.95 A			
ZnO:Mn	0.2	0.4	0.8		*MnO
	0.70 A*+	0.00 B+	0.75 A*+		0.00
ZnO:Mo	0.5	1.0	5.0	10.0	
	0.79 B+	0.91 A+	0.00 C+	0.81 B+	
ZnO	0.00				
ZnOCl	0.00				
Copper hydroxide	1.10				
Cephalexin	1.02				
H_2O	0.00				

Data are means of three replicates. Significant differences among concentrations, using Tukey's test ($P < 0.05$), are indicated by letters. Significant differences between copper hydroxide group and pure NPs, using Dunnett's test ($P < 0.05$), are indicated by * and +. .

Table 3: Growth inhibition zones (cm) of different nanoparticles against *Pantoea ananatis* at 10 mg mL^{-1}.

Doped NPs	Concentrations of doping agents			
ZnO:K	0.5	1	5	10
	0.00 B	1.00 A+	0.00 B	0.90 A+
ZnO:Mo	0.5	1	5	10
	1.10 B+	1.30 A+	0.00 C	0.00 C
ZnO	0.00			
ZnOCl	0.00			
Copper hydroxide	0.00			
Streptomycin	1.16			
H_2O	0.00			

Data are means of three replicates. Significant differences among concentrations, using Tukey's test ($P < 0.05$), are indicated by letters. Significant differences between copper hydroxide group and pure NPs, using Dunnett's test ($P < 0.05$), are indicated by * and +.

Table 4: Growth inhibition zones (cm) of different nanoparticles against *Xanthomonas vasicola* pv. *vasculorum* at 10 mg mL^{-1}.

Doped NPs	Concentrations of doping agents				Pure NPs
ZnO:Ag	0.7	1	9	11	*Ag O_2
	0.90 A*+	1.15 A+	1.20 A+	0.90 A*+	1.45
ZnOCl:Ag	0.1	5			
	0.00 B*	0.85 A*+			
ZnO:Fe	0.5	1	5		*FeO
	0.00 B	0.90 A*+	0.00 B		0.00
ZnO	0.00				
ZnOCl	0.00				
Copper hydroxide	0.00				
Streptomycin	1.12				
H_2O	0.00				

Data are means of three replicates. Significant differences among concentrations, using Tukey's test ($P < 0.05$), are indicated by letters. Significant differences between copper hydroxide group and pure NPs, using Dunnett's test ($P < 0.05$), are indicated by * and +.

In contrast, doped NPs, antibiotic cephalexin, and copper hydroxide significantly inhibited *D. zeae* growth compared to water (negative control), with inhibition zones ranging from 0.7 to 1.10 cm (Table 2). Among the pure NPs, Ag_2O, CuO, and NiO NPs significantly inhibited *D. zeae* growth, with a 0.80 cm inhibition zone. Ag-doped ZnO (ZnO:1Ag and ZnO:9Ag) and Ag-doped ZnOCl (ZnOCl:1Ag, ZnOCl:10Ag) NPs had similar bacterial inhibitory effects as the pure Ag_2O NPs (0.80 cm), indicating that Ag doping improved the performance of ZnO and ZnOCl NPs. Only ZnO:0.5K and ZnO:1Mg NPs had similar bacterial inhibitory effects (0.95 cm) as copper hydroxide (1.10 cm). Ag NPs are the most explored and widely used microorganism control agents due to the high antimicrobial activity of silver ions (Antunes et al., 2013).

Additionally, ZnO:1K, ZnO:10K, ZnO0.5Mo, and ZnO:1Mo NPs significantly inhibited *P. ananatis* growth, with inhibition zones ranging from 0.9–1.3 cm (Table 3). As expected, water (negative control) did not inhibit bacterial growth, whereas streptomycin (positive control) significantly inhibited *P. ananatis* growth (1.16 cm). Among the NPs, ZnO:1K and ZnO:1Mo had the highest bactericidal effects, with inhibition zones of 1.00 and 1.30 cm, respectively. Previously tests with ZnO NPs doped with Ag, Cu, Fe, Mn, and Ni; and pure NPs of Ag_2O, CuO, and NiO were performed *in vitro* and inhibited bacterial growth (Mamede, 2022).

ZnO:0.7Ag, ZnO:1Ag, ZnO:9Ag, ZnO:11Ag, ZnOCl:5Ag, ZnO:1Fe, and Ag_2O NPs significantly inhibited *X. vasicola* pv. *vasculorum* growth, with inhibition zones ranging from 0.85–1.45 (Table 4). As expected, water (negative control) did not inhibit *X. vasicola* pv. *vasculorum* growth and streptomycin (positive control) did (1.12 cm). Among the NPs, ZnO:1Ag and ZnO:9Ag had the highest inhibitory effect, with inhibition zones of 1.15 and 1.20 cm, respectively, which were similar to that of pure AgO NP (1.45 cm). Despite forming an inhibition zone (0.85 cm), ZnOCl:5Ag NP had a lower antibacterial activity than ZnO:Ag NPs at the different concentrations tested.

Copper hydroxide did not inhibit the growth of *P. ananatis* and *X. vasicola* pv. *vasculorum*, but significantly inhibited the growth of *D. zeae*. The isolates of *P. ananatis* and *X. vasicola* pv. *vasculorum* may be resistant to copper hydroxide. Moreover, the toxicity of copper hydroxide could be altered despite penetrating the culture medium and bacterial cells, which may have reduced its effect on the bacteria. Among the NPs, ZnO:Ag (Figure 1C), ZnO:K, ZnO:Mo (Figure 1B), ZnOCl:Ag, Ag_2O, CuO, and NiO (Figure 1A) showed significant inhibitory effects against the three bacterial species. Additionally, the bacterial inhibitory effect was not significantly influenced by an increase in the concentration of the doping agents for most NPs, as evidenced by the relatively similar inhibitory zones at different concentrations.

Lower concentrations of the doping agents may result in decreased aggregation of the NPs and, consequently, increased reactive surface area of NPs with the microorganism, improving bactericidal activity (Fraga et al., 2021). Czyżowska and Barbasz (2020) reported that the antibacterial activity of NPs is mainly dependent on their synthesis method and the effects of the resulting surface fillers.

The effectiveness of NPs is dependent on the production of ROS, as the higher the amount of ROS produced by the compounds, the higher their antibacterial activity (Lin et al., 2017; Ning et al., 2017). The radical ROS superoxide

(O_2^-), hydroxyl (OH^-), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) can directly attack cell membranes and inactivate bacteria or accumulate in the cytoplasm (Czyzowska & Barbasz, 2020).

Preventive and curative applications of NPs to control WS and BLS in maize

In both trials, preventive and curative applications of the different NPs had varying effects against WS and BLS (Tables 5 and 6), as evidenced by the AUDPC.

For WS, no significant interaction was observed between the NPs and each application (preventive or curative) (Tables 5 and 6) in both trials. In trial 1, the curative application of NPs caused a decrease in WS severity(average AUDPC = 4.29). In trial 2, there was no significant difference in the average AUDPC between the preventive (11.87) and curative (11.88) applications. Compared with that of water (AUDPC = 9.90), the application of ZnO:1.0Ag (6.19) and ZnO:9.0Ag (5.87) NPs and NiO (6.13) NP reduced WS severity in trials 1 and 2, respectively. Mamede et al. (2022) applying ZnO NPs doped with Cu and Mn in different concentrations (2.5; 5.0 and 10 mg mL-1) also reduced the severity of WS in preventive and curative applications.

Discrepancies in the AUDPC for WS following copper application in trials 1 (4.95) and 2 (20.59) were found, which could be attributed to phytotoxicity. Notably, leaf burning was observed in trial 1, which made the disease evaluation difficult. Although leaf burning was not observed in trial 2, the severity of the disease was higher (20.59) than those of the control (9.90), ZnO:9.0Ag (9.49), and NiO (5.36) NPs, following preventive application. A previous study has shown that copper hydroxide- and copper oxychloride-based products for *P. ananatis* control exhibit phytotoxic effects in maize plants (Bomfeti et al., 2007). Overall, these results suggest that copper application at recommended levels can allow the entry and establishment of the bacterium, increasing the severity of WS.

For BLS, a significant interaction between the NP used and the application method was found only in trial 2 (Tables 5 and 6). In trial 1, the preventive application of NPs decreased BLS severity, with an average AUDPC = 160.88; additionally, the application of NiO (90.56) and ZnO (141.75) NPs reduced the severity of the disease. Interestingly, preventive (203.25) and curative (256.50) applications of the NiO NP reduced BLS severity in trial 2 compared with the control group (333.00).

In contrast, the curative application of ZnO NP increased BLS severity (603.00) in trial 2 compared to the control (333.00). Despite the increase in BLS severity following preventive applications of the NPs, curative application of ZnO:Ag NPs (0.7; 1.0; and 9.0), especially ZnO:0.7Ag NPs (218.63) reduced LBS severity. Ocsoy et al. (2013) reported that the application of Ag NPs at low concentrations was effective against *Xanthomonas perforans* in tomato and significantly reduced the severity of tomato bacterial spot in the greenhouse. Furthermore, copper application reduced the severity of BLS in both trials, with AUDPC of 151.50 and 280.88 in trials 1 and 2, respectively. Fraga et al. (2021) observed a decrease in tomato bacterial spot (*X. gardneri*) severity after copper hydroxide application. Here, most of the NPs examined, except ZnO:0.7Ag (338.25) in trial 1 and ZnO:1.0Ag (569.25) and ZnO:9.0Ag (468.00) in trial 2, could serve as alternatives to copper hydroxide for BLS control.

Interestingly, *P. ananatis* and *X. vasicola* pv. *vasculorum* showed reduced disease severity (especially BLS) when inoculated together compared with individual inoculation (Tables 5 and 6). Although there was no significant difference between curative and preventive applications of the NPs on WS + BLS severity in trial 2, curative application of the NPs (5.94) reduced the severity of WS + BLS in trial 1. All NPs reduced WS severity in trials 1 and 2, whereas the least mean disease severity in trial 2 was obtained for NiO NP (5.78).

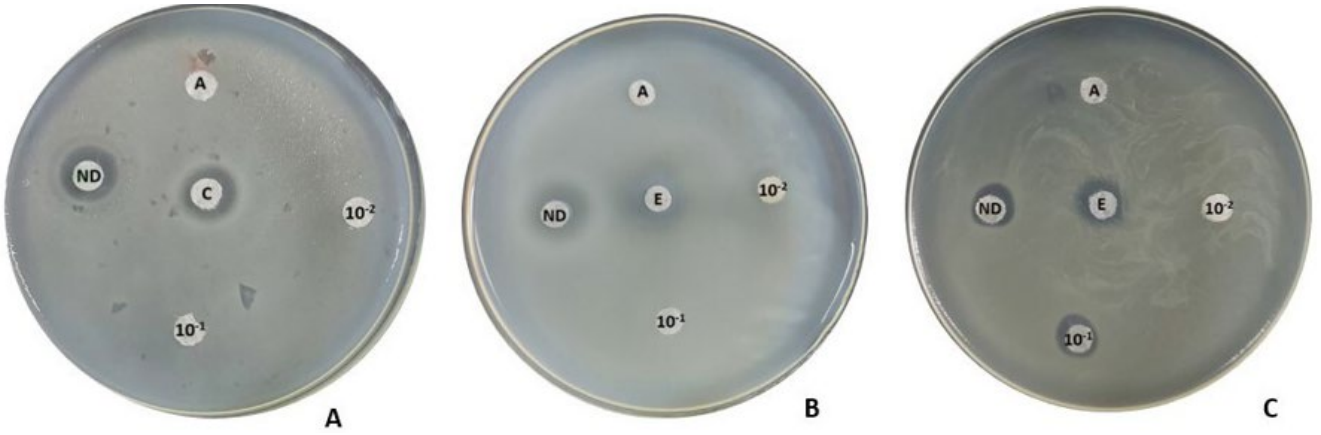

Figure 1: (A) Inhibition halo of NiO NPs in *D. zeae* (B) Inhibition halo of ZnO:Mo NPs in *P. ananatis* (C) Inhibition halo of ZnO:Ag NPs in *X. vasicola* pv. *vasculorum.*

Table 5: Area under the disease progress curve for white spot and bacterial leaf streak using different nanoparticles (NPs) in preventive and curative applications, trial 1.

Disease	Application	NPs					Average
		ZnO:0.7Ag	ZnO:1Ag	ZnO:9Ag	NiO	ZnO	
WS	Preventive	18.53*	9.90	9.68	15.41	14.40	13.58B
	Curative	2.48	2.48	2.06	9.49	4.95	4.29A
	Average	10.50a	6.19a	5.87a	12.45a	9.68a	
	Copper*	4.95					
	Control+	9.90					
BLS	Preventive	237.00+	180.00+	213.00+	70.88+	103.50+	160.88A
	Curative	338.25*	237.00+	180.00+	110.25+	180.00+	209.10B
	Average	287.63c	208.50b	196.50b	90.56a	141.75a	
	Copper*	151.50					
	Control+	372.00					
WS +	Preventive	11.18	7.76	9.90	17.36	9.90	11.22B
	Curative	4.95	4.95	4.95	4.95	9.90	5.94A
	Average	8.06a	6.36a	7.43a	11.16a	9.90a	
	Copper*	4.95					
	Control+	17.40					
BLS	Preventive	21.94Aa	16.69Aa	28.50Aa	9.94Aa	17.25Aa	18.86
	Curative	44.63Aa	24.56Aa	22.31Aa	21.75Aa	97.50Bb*+	42.15
	Average	33.28	20.63	25.41	15.84	57.38	
	Copper*	24.56					
	Control+	37.50					

CV% (WS) = 60.87; CV% (BLS) = 28.89; CV% (WS + BLS) = 76.36 and 59.69. Data are means of three replicates. Significant differences among NPs in the same disease and treatments (curative or preventive), using Scott–Knott test ($P < 0.05$), are indicated by upper case and lower case letters, respectively. Significant differences between each NP and the commercial product or water (control), using Dunnett's test ($P < 0.05$), are shown by * and +, respectively.

For *X. vasicola* pv. *Vasculorum*, all NPs reduced the severity of BLS in trial 1, except the curative application of ZnO NP (97.50), which increased severity compared with the control (37.50). In trial 2, disease severity was lower after curative application (74.01) regardless of the NP used. Copper reduced BLS severity in both trials (24.56 and 59.25); however, there was an increase in WS severity (11.03) in trial 2. Overall, this result confirms that copper application reduces BLS but increases WS severity.

In cultivated maize plants, leaf spots are widely distributed diseases that cause crop losses and decrease productivity. This is due tothe malfunction of photosynthetic cells and tissues and the subsequent expansion of the necrotic leaf area (Agrios, 2005). Generally, the symptoms of these diseases are clearly visible and can change considerably depending on the causal agent (Amorim, Rezende, & Bergamin Filho, 2018). However, it is not always possible to visualize the signs of pathogens, and some symptoms may be confusing depending on the causal agent (Agrios, 2005). For instance, both *P. ananatis* and *X. vasicola* pv. *vasculorum* can penetrate the host via natural openings, such as stomata or wounds, and their symptoms can be observed on young leaves at the initial stages and on the bracts and cobs at advanced stages (Oliveira et al., 2004; Leite Jr et al., 2018).

Metallic NPs have been reported to show beneficial effects in plants by activating defense mechanisms and micronutrient uptake (Cartwright et al., 2020). The concentration and particle size used must be considered when spraying NPs (Mamede et al., 2022). Overall, the results of our study showed for the first time that ZnO NPs doped with Ag at different concentrations and NiO NPs reduced the severity of WS and BLS and can be used in the management of diseases.

Table 6: Area under the disease progress curve for white spot and bacterial leaf streak using different nanoparticles (NPs) in preventive and curative applications, trial 2.

Disease	Application	NPs					Average
		ZnO:0.7Ag	ZnO:1Ag	ZnO:9Ag	NiO	ZnO	
WS	Preventive	16.65	17.96	9.49*	5.36*	9.90	11.87A
	Curative	14.78	9.90	13.65	6.90*	14.18	11.88A
	Average	15.71b	13.93b	11.57b	6.13a	12.04b	
	Copper*	20.59					
	Control+	9.90					
BLS	Preventive	372.00Ba	569.25Bb*+	468.00Bb*	203.25Aa	304.50Aa	383.40
	Curative	218.63Aa	251.25Aa	306.00Aa	256.50Aa	603.00Bb*+	327.08
	Average	295.31	410.25	387.00	229.88	453.75	
	Copper*	280.88					
	Control+	333.00					
WS +	Preventive	8.55	11.03	12.90	6.60	9.90	9.80A
	Curative	9.45	7.43	7.43	4.95	9.90	7.83A
	Average	9.00a	9.23a	10.16a	5.78a	9.90a	
	Copper*	11.03					
	Control+	9.90					
BLS	Preventive	70.88	208.13	174.94	153.56	99.37	141.37B
	Curative	71.63	102.75	44.06	63.56	88.06	74.01A
	Average	71.25a	155.44a	109.50a	108.56a	93.72a	
	Copper*	59.25					
	Control+	78.75					

CV% (WS) = 44.99; CV% (BLS) = 25.74; CV% (WS + BLS) = 45.34 and 77.10. Data are means of three replicates. Significant differences among NPs in the same disease and treatments (curative or preventive), using Scott–Knott test ($P < 0.05$), are indicated by upper case and lower case letters, respectively. Significant differences between each NP and the commercial product or water (control), using Dunnett's test ($P < 0.05$), are shown by * and +, respectively.

Conclusions

This is the first report of pure NPs (ZnO, ZnOCl, Ag2O, CuO, FeO, MnO, and NiO), doped NPs (ZnO:Ag, ZnO:Cu, ZnO:Fe, ZnO:K, ZnO:Mg, ZnO: Mn, ZnO:Mo, and ZnO:Ni), and simonkolleite NPs (ZnOCl:Ag and ZnOCl:Cu) for the control of *D. zeae* and *X. vasicola* pv. *vasculorum in vitro*, and ZnO:K, ZnO:Mo, ZnOCl, ZnOCl:Ag, and ZnOCl:Cu for the control of *P. ananatis*.

Author Contribution

Conceptual idea: Mamede, M. C.; Tebaldi, N.D.; Methodology design: Mamede, M. C.; Tebaldi, N.D.; Data collection: Mamede, M. C.; Data analysis and interpretation: Mamede, M. C.; Tebaldi, N.D.; Writing and editing: Mamede, M. C.; Tebaldi, N.D.; Silva, A.C.A.

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