Eur J Plant Pathol
https://doi.org/10.1007/s10658-025-03140-y

Effects of zinc oxide submicron particles and nanoparticles on the inhibition of *Botrytis cinerea*, *Fusarium oxysporum* and *Alternaria alternata*

Jolanta Kowalska · Małgorzata Antkowiak · Joanna Krzymińska · Urszula Szałaj · Jacek Wojnarowicz · Alicja Tymoszuk · Małgorzata Jeske · Aleksander Łukanowski · Dariusz Pańka

Received: 17 December 2024 / Accepted: 24 September 2025

Abstract Zinc oxide nanoparticles (ZnO NPs) exhibit antifungal properties and may be applied for limitation of pathogen growth in crop production. The objectives of the study were: 1) to investigate and compare the effects of ZnO submicron particles (ZnO SMPs) and ZnO nanoparticles (ZnO NPs) on the *in vitro* response of *Botrytis cinerea*, *Fusarium oxysporum,* and *Alternaria alternata* and 2) to evaluate the impact of those particles on suppressing disease symptoms caused by the three plant pathogens on potted tomato plants (*Solanum lycopersicum* L. 'Bawole Serce'). In the *in vitro* experiment ZnO SMPs or ZnO NPs at the concentration of 0, 100, 200, 500, 1000, and 2000 mg/l were applied. In the *in vivo* experiment, potted tomato plants were infected with pathogens and sprayed with ZnO SMPs or ZnO NPs suspensions at the concentration of 500 mg/l. Experimental objects included also non-infected control plants, as well as infected and ZnO SMPs/NPs non-treated control plants. The addition of ZnO NPs and ZnO SMPs to the PDA medium significantly inhibited the growth of mycelium of all tested pathogens. However, the advantage of ZnO NPs over ZnO SMPs in inhibiting the growth of mycelium has not been demonstrated. Interestingly, the lowest tested ZnO SMPs/NPs concentration (100 mg/l) caused a significant reduction of mycelium growth of *F. oxysporum* and *A. alternata* (up to 33.32%). As for *B. cinerea*, only the concentration of 500 mg/l and higher concentrations of tested material samples limited the growth of its mycelium (up to 82.67%). *In vivo* experiment on tomato plants did not confirm the effectiveness of ZnO SMPs in the reduction of *B. cinerea* and *A. alternata* infection, in contrast to ZnO NPs, which significantly limited the growth of all pathogens (by between 56.99% and 65.11%).

Keywords *Alternaria alternata* · Antifungal activity · *Botrytis cinerea* · *Fusarium oxysporum* · Nanotechnology · Zinc oxide

J. Kowalska · M. Antkowiak · J. Krzymińska
Department of Organic Agriculture and Environmental Protection, Institute of Plant Protection – National Research Institute, Władysława Węgorka 20, 60-318 Poznan, Poland

U. Szałaj · J. Wojnarowicz
Laboratory of Nanostructures, Institute of High Pressure Physics, Polish Academy of Sciences, Sokolowska 29/37, 01-142 Warsaw, Poland

A. Tymoszuk (✉)
Laboratory of Horticulture and Landscape Architecture, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Bernardynska 6, 85-029 Bydgoszcz, Poland
e-mail: alicja.tymoszuk@pbs.edu.pl

M. Jeske · A. Łukanowski · D. Pańka
Department of Microbiology and Plant Ecology, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Kaliskiego 7, 85-796 Bydgoszcz, Poland

Published online: 09 October 2025

Introduction

Nanoparticles (NPs), due to their small size, show some specific physicochemical properties, which often enable them to be used in new applications. Over the past few decades, nanotechnology has significantly developed and become a part of our everyday life. Nanoparticles are expected to be used in agriculture as fertilizers, growth promoters, and pesticides. Recent research also focuses on the antifungal properties and protective capabilities of nanoparticles (Pan et al., 2022).

The size of particles can significantly influence their physical, chemical, and biological properties. Submicron particles (SMPs) generally have sizes between 100 and 1000 nm, whereas nanoparticles (NPs) are defined as materials with at least one dimension between 1 and 100 nm. These differences in size affect their surface area, reactivity, and interaction with biological systems. Nanoparticles exhibit a higher surface-to-volume ratio, which enhances their reactivity, bioavailability, and potential antimicrobial activity. Their smaller size allows them to penetrate microbial cell membranes more effectively, disrupting cellular structures and metabolic pathways. In contrast, submicron particles, due to their larger size, have reduced bioactivity and are typically less effective at inhibiting pathogens at lower concentrations. However, ZnO SMPs may still be effective due to their slow dissolution, acting as a sustained-release source of Zn^{2+} ions, which contribute to antifungal activity (Mgadi et al., 2024).

Zinc oxide nanoparticles (ZnO NPs) are one of the most frequently produced nanoparticles in the world. Due to their unique physical and chemical properties, they are considered valuable and versatile inorganic compounds with a wide range of applications. They are characterized by an extended absorption spectrum, a strong electrochemical coupling coefficient and high chemical stability. ZnO NPs are incorporated into various commercial products, including plastics, glass, ceramics, cement, ointments, adhesives, sealants, pigments, batteries, cosmetics and sunscreens as well as used as a source of zinc in food products. Due to their biocompatibility, antibacterial properties and biodegradability, ZnO NPs are of great interest in biomedicine (Wojnarowicz et at. 2020). Zinc plays a key role in various metabolic processes, plant growth and development. ZnO NPs, as compared to other nanoparticle types, are safe for plants and less toxic to beneficial soil bacteria. They are used in agricultural production to support seed germination and plant growth (Dimkpa et al., 2013; Tymoszuk & Wojnarowicz, 2020).

Plant pathogens have a large impact on agricultural production. It is estimated that plant diseases reduce the yield of crops worldwide by 21–30%. Fungicides are widely used to control fungal pathogens in crop production. However, numerous pathogens have developed resistance to certain chemical antifungal compounds. Currently used control strategies are often insufficient. There is an urgent need to develop new and effective means to control infections caused by resistant fungal pathogens (El-Saadony et al., 2022). One of the most common plant pathogens are *B. cinerea*, *F. oxysporum* and *A. alternata*. The causal agent of grey mold, *B. cinerea,* can infect over 200 crop species worldwide, causing significant losses, particularly in mature and senescent dicotyledonous plant tissues. Its enormous enzymatic activity results in yield losses in the field and greenhouse, as well as during crop storage. Diverse modes of infection make *B. cinerea* difficult to control. *F. oxysporum* infects many economically important plants, causing vascular disease and Fusarium wilt. Its spores can survive in a dormant state in the soil for up to several dozen years; thus, it is difficult to control. *A. alternata* is both an endophyte and a plant pathogen, growing on over 380 plant species, including many key crops. Alternaria black spot symptoms usually develop late during cold storage, leading to significant post-harvest losses (Kowalska et al., 2025).

Chemical control is not effective enough because of the decreasing number of conventional active compounds, among others due to strict environmental safety regulations, e.g. metiram, famoxadone used for control of tomato diseases, which were withdrawn in the EU. In this context, metal-containing nanoparticles (MNPs), such as ZnO NPs, are promising alternatives to traditional fungicides, offering a valuable strategy for managing plant pathogen resistance. ZnO NPs exhibit a wide range of antibacterial and antifungal properties that may be beneficial in the control of various plant pathogens. These nanoparticles are not only stable but also have a longer shelf life as compared to traditional organic disinfectants and are widely recognized as safe for human health. However, the antifungal effectiveness of ZnO NPs is not

researched so much as their antibacterial properties (Dimkpa et al., 2013; Kairyte et al., 2013; Malandrakis et al., 2022).

This study aimed to investigate and compare the effect of ZnO submicron particles (ZnO SMPs) and ZnO nanoparticles (ZnO NPs) on the response of three plant pathogens, i.e. *B. cinerea*, *F. oxysporum* and *A. alternata* in *in vitro* culture conditions and in *in vivo* experiment with infected tomato (*S. lycopersicum*) plants. ZnO NPs were expected to exhibit stronger antifungal activity against *B. cinerea*, *F. oxysporum* and *A. alternata* compared to ZnO SMPs in both *in vitro* and *in vivo* conditions, potentially reducing disease severity in infected tomato plants. Many recent studies focus solely on ZnO NPs particles' synthesis and *in vitro* properties or on a single plant pathogen. Our study compares ZnO SMPs and ZnO NPs in both *in vitro* and *in vivo* conditions, highlighting differences in antifungal efficacy and evaluating the potential real-world application of ZnO NPs as a plant protection agent. It targets three major plant pathogens and assesses their response to ZnO particle treatments — providing insights into pathogen-specific effectiveness.

Materials and methods

Materials used in the synthesis of zinc oxide samples

Microwave solvothermal synthesis was applied to produce ZnO NPs (Tymoszuk & Wojnarowicz, 2020). Zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$, Avantor Performance Materials Poland S.A., Gliwice, Poland), ethylene glycol ($C_2H_4(OH)_2$, Chempur, Piekary Śląskie, Poland), and deionized water (H_2O) were used for the ZnO NPs synthesis. ZnO SMPs produced in the indirect French process, were purchased from ZM SILESIA SA, Huta Oława, Oława, Poland (Oława, 2024) and used as a reference sample.

Characterization of zinc oxide samples

The description of the test procedures can be found in our previous paper (Tymoszuk & Wojnarowicz, 2020). The tests were carried out in accordance with PN-EN ISO/IEC 17025:2018–02 (2023) in the Laboratory of Nanostructures (IHPP PAS, Warsaw, Poland) with accreditation no. AB 1503 (Polish Center for Accreditation, Testing Laboratories, 2022).

Morphology of ZnO powders samples were obtained using a scanning electron microscope ULTRA PLUS (ZEISS, Oberkochen, Germany). The skeleton density of ZnO samples was tested in accordance with ISO 12154:2014 standard (AccuPyc II 1340 helium pycnometer, Micromeritics®, Norcross, GA, USA). The specific surface area of ZnO samples was tested in accordance with ISO 9277:2010 standard (Brunauer–Emmett–Teller (BET) method, Gemini 2360 surface analyser, Micromeritics®, Norcross, GA, USA). The phase composition of ZnO samples was analysed by the X-ray diffraction (XRD) method using an X'Pert PRO diffractometer (CuKα, Panalytical, Almelo, The Netherlands). The size of the crystallites was calculated using the Scherrer formula.

ZnO samples for Energy Dispersive Spectrometry (EDS) measurement were carbon sputtered. The samples were not compressed into a pellet form to avoid introducing contamination from the mould. The X-ray microanalysis was carried out using the EDS analyser (Quantax 400, Bruker, USA). Prior to EDS analysis, the samples were sputter-coated with a thin film of carbon (SCD 005/CEA 035 Cool Sputter Coater, BAL-TEC, Switzerland). The measurement was repeated 6 times for each sample. The quantitative EDS results presented in this study are arithmetic means with standard deviation from six measurements. The results were determined with the use of Excel 365 software (Microsoft Corporation, Redmond, WA, USA).

The average particles size was calculated based on the results of skeleton density and specific surface area with the assumption that the tested samples contained only spherical and identical.

The SEM images were used to determine the particle size distribution. Data collection consisted of graphical analysis of the size of individual particles. The determination of the size of the individual particles consisted of calculating the diameter of the circle described around each particle. The diameters were determined with the use of ImageJ 1.52a (Wayne Rasband, National Institutes of Health, USA) and Excel 365 software (Microsoft Corporation, Redmond, WA,

USA). The diameters were determined for a minimum of 220 particles in each sample and a histogram was created (a bar graph of the number of particles with diameters in the specified range). The average particle size was provided as an average calculated with the use of OriginPro 6.1 software (Northampton, MA, USA), using log-linear distribution curve fitting.

In vitro experiment

Plant pathogens were obtained from the collection of the Bank of Pathogens of Institute of Plant Protection—National Research Institute (Poznan, Poland). Their origin was as follows: *B. cinerea* 2235—tomato stem (*Solanum lycopersicum* L.) grown in Chwaszczowo, Poland, and stored in glycerol at −20 °C; *F. oxysporum* 2105—*Triticum vulgare* Vill. grown in Winna Góra, Poland, and preserved in liquid nitrogen; *A. alternata* 2115—tomato leaves (*Solanum lycopersicum* L.) collected in Plewiska, Poland, and stored in liquid nitrogen. All pathogens were inoculated on the Potato Dextrose Agar (PDA) medium (BD Difco™, New Jersey, USA) with the addition of ZnO SMPs or ZnO NPs at the following concentrations: 0, 100, 200, 500, 1000, 2000 mg/l. ZnO SMPs and ZnO NPs were added into the PDA medium, and such obtained suspensions were sterilized in an autoclave at 121 °C and 1 atm for 15 min, and next placed for 30 min in the Elmasonic S80(H) Ultrasonic Cleaner (37 kHz, 150 W; Elma Schmidbauer GmbH, Singen, Germany) for proper particles dispersion. Then media were immediately poured (20 ml) onto 90 mm Petri dishes. Fresh discs of seven-day-old pathogens cultures: *B. cinerea*, *F. oxysporum,* and *A. alternata* (5 mm diameter) were cut and separately transferred onto a medium (one disc in the center of each Petri dish; five Petri dishes per each pathogen). Cultures were then incubated at 23 °C in darkness. The diameter (mm) of mycelium was measured every 24 h until it reached the edge of the dish in one of the treatments (for each pathogen separately). The growth dynamics were created.

In vivo experiment

Solanum lycopersicum L. cultivar 'Bawole Serce' seeds (PlantiCo Ltd., Zielonki Parcela, Poland) were individually sown into plastic pots (0.25 l, 7×7×9 cm) filled with universal horticultural substrate (Hartmann Polska Sp. z o.o. Sp. K., Poznan, Poland) on December 17, 2023. Pots with seeds were maintained in a natural photoperiod in a glasshouse (53°07′12.0"N 18°00′29.4"E). After two months, pots with plants were transferred to a growth chamber with a controlled environment (20 °C; relative air humidity of 80–90%; 12/12-h day and night cycle, light source – LED TUBE LIGHT—For Plant Growing T8 SKU6326 RED, V-TAC Poland sp. z o.o., Złotniki, Poland).

The experiment consisted of four treatments: (1) non-infected control plants (negative control); (2) infected control plants (positive control); (3) infected and 500 mg/l ZnO SMPs sprayed plant; (4) infected and 500 mg/l ZnO NPs sprayed plants. Each treatment included 8 potted tomato plants. The concentration of ZnO SMPs and ZnO NPs suspensions was chosen based on the *in vitro* experiment results. The plants chosen for testing were healthy, without any symptoms of diseases or the presence of pests.

For plant inoculation with pathogens, suspension prepared from conidial spores and mycelial hyphae of two-week-old cultures of the tested pathogens grown on the PDA medium was used. Appropriate concentrations of the inoculation suspension were prepared (2.0×10^{-6} CFU/ml) using a Thoma hemocytometer. Inoculation was performed using a pressure applicator. Twenty four hours before plant inoculation with *B. cinerea*, *F. oxysporum* and *A. alternata*, the first preventive foliar spraying with 500 mg/l ZnO SMPs and ZnO NPs suspensions (treatments 3, 4) was carried out. Next, the plants were inoculated with pathogens (treatments 2–4).

Fourteen days after inoculation, the first evaluation of plant health was conducted. The percentage of diseased leaf area of plants was visually evaluated according to EPPO Standard PP 1/121(2).

Then, a second spraying with the tested ZnO SMPs and ZnO NPs suspensions was performed, and after 24 h, a second plant infection was performed with *B. cinerea*, *F. oxysporum* and *A. alternata* inoculum suspensions. Another 14 days after infection, the second evaluation of plant health was conducted.

Statistical analysis

The results were statistically processed using the Statistica 12.0 program (StatSoft Polska, Cracow,

Poland). Prior to the analysis, the Shapiro–Wilk test for normal distribution was performed. For normally distributed data, the results were statistically processed by two-way analysis of variance (ANOVA) and a post hoc Fisher test at the significance level of $p \leq 0.05$. Data expressed as a percentage underwent the Freeman-Tukey transformation prior to ANOVA verification. Experimental treatments consisted of 5 (*in vitro*) and 8 (*in vivo*) repetitions.

Results

Properties of zinc oxide samples

The ZnO SMPs sample contained heterogeneous particles in terms of shape and size (Fig. 1). The ZnO SMPs sample consisted of hexagonal rods, spherical particles and heterogeneous structures (Fig. 1A-C). The SEM image (Fig. 1C) of the ZnO SMPs sample shows the particle aggregates. The average

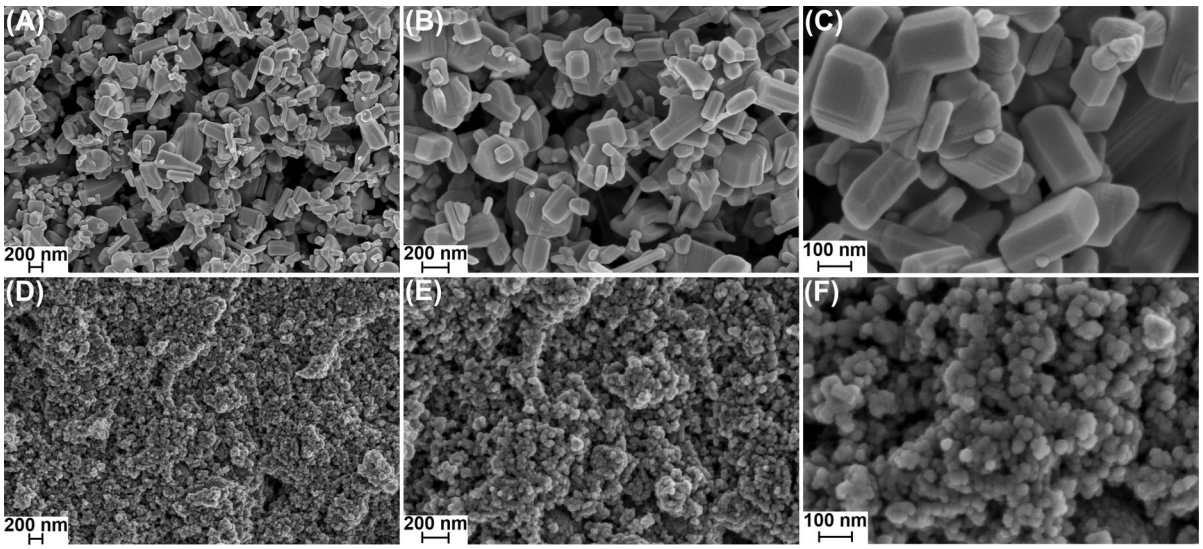

Fig. 1 Scanning electron microscopy images of zinc oxide samples: zinc oxide submicron particles (ZnO SMPs) (**A**, **B**, **C**) and zinc oxide nanoparticles (ZnO NPs) (**D**, **E**, **F**)

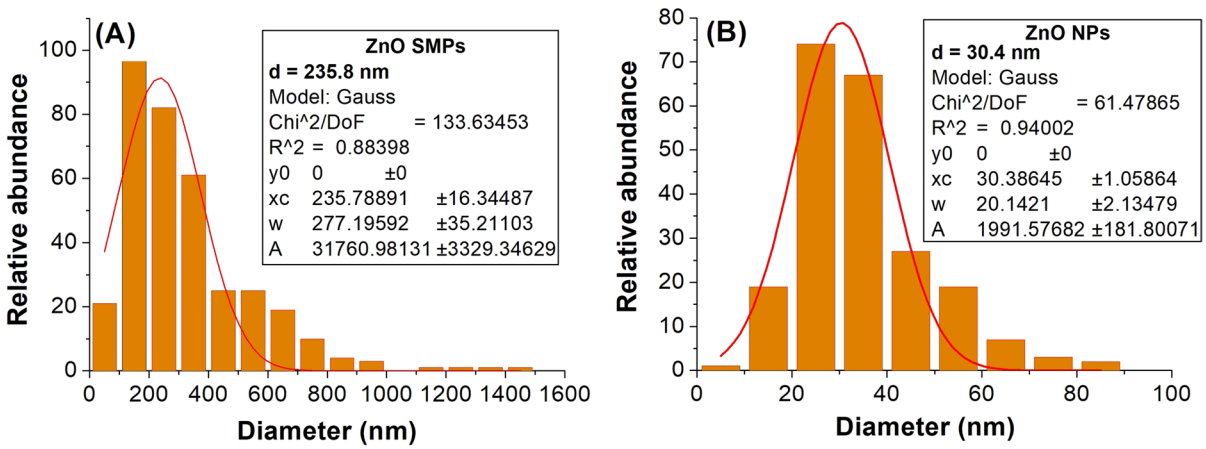

Fig. 2 Particle size distribution of zinc oxide samples: zinc oxide submicron particles (ZnO SMPs) (**A**) and zinc oxide nanoparticles (ZnO NPs) (**B**)

size calculated from the SEM images was 235.8 nm and the size distribution ranges from 40 to 1700 nm (Fig. 1A). The sample ZnO NPs consisted of particles homogeneous in shape and size (Fig. 1D-F). ZnO NPs were characterised by spherical particle shape (Fig. 1F) with an average size of 30.4 nm and a size distribution ranging from 9 to 90 nm (Fig. 2B).

No foreign phases were detected in the ZnO NPs samples. All diffraction peaks shown in Fig. 3 are from the hexagonal ZnO phase, which is described in JCPDS card No. 36–1451 (results of the reference sample). Figure 3 shows the differences between the widths of the diffraction peaks of the ZnO phase, which is related to the different sizes of the crystallites. The size of the crystallites of ZnO SMPs and ZnO NPs were 27 nm and 124 nm, respectively. The results of the EDS analysis of the ZnO samples showed no traces of heavy metals (Fig. 4). Quantitative analysis showed that the ZnO NPs sample contained an average of 56.3 ± 4.3 atom % zinc and 43.7 ± 4.3 atom % oxygen, and for the ZnO SMPs sample, the average results were 56.9 ± 2.2 atom % zinc and 43.0 ± 2.2 atom % oxygen content.

The characteristics of the samples are included in the Table 1. The sample density of ZnO SMPs was higher than that of ZnO NPs which was related to their particle size. As the size of ZnO particles increases, their density increases and their specific surface area decreases. The average crystallite size of the ZnO NPs was identical to the average particle size which means that the ZnO particles were made of a single crystallite (monocrystalline structure). The average particle size of ZnO SMPs was twice the size of the crystallites, which means that one ZnO SMPs particle was built from two crystallites (polycrystalline structure).

Effects of zinc oxide submicron particles and zinc oxide nanoparticles on the inhibition of *B. cinerea*

Pathogen completely overgrown the Petri plate in just four days on medium with the addition of ZnO SMPs and ZnO NPs, at a concentration of 100 mg/l. The growth dynamics were the highest between the second and fourth day, starting from the control to a concentration of 500 mg/l for ZnO SMPs and ZnO NPs. When the medium was modified with 1000 mg/l ZnO SMPs/ZnO NPs, the pathogen grew the fastest between the first and second day, then the growth dynamic was slightly reduced. When the highest concentration (2000 mg/l) of the tested zinc oxide samples was used, the pathogen grew most intensively in the last 24 h (Fig. 5A-B). The growth of *B. cinerea* mycelium was inhibited by ZnO SMPs at concentrations of 200—2000 mg/l. When the pathogen was treated with ZnO NPs, the concentrations of 100 and 200 mg/l turned out to be insufficient to inhibit the mycelium growth as compared to the control.

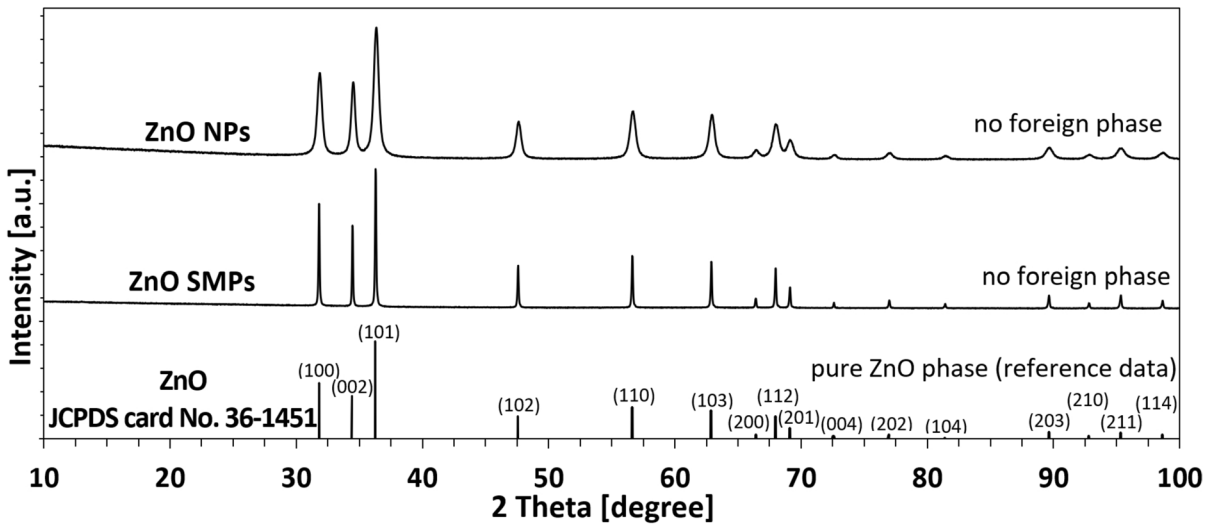

Fig. 3 X-ray diffraction patterns of zinc oxide samples: zinc oxide nanoparticles (ZnO NPs) and zinc oxide submicron particles (ZnO SMPs)

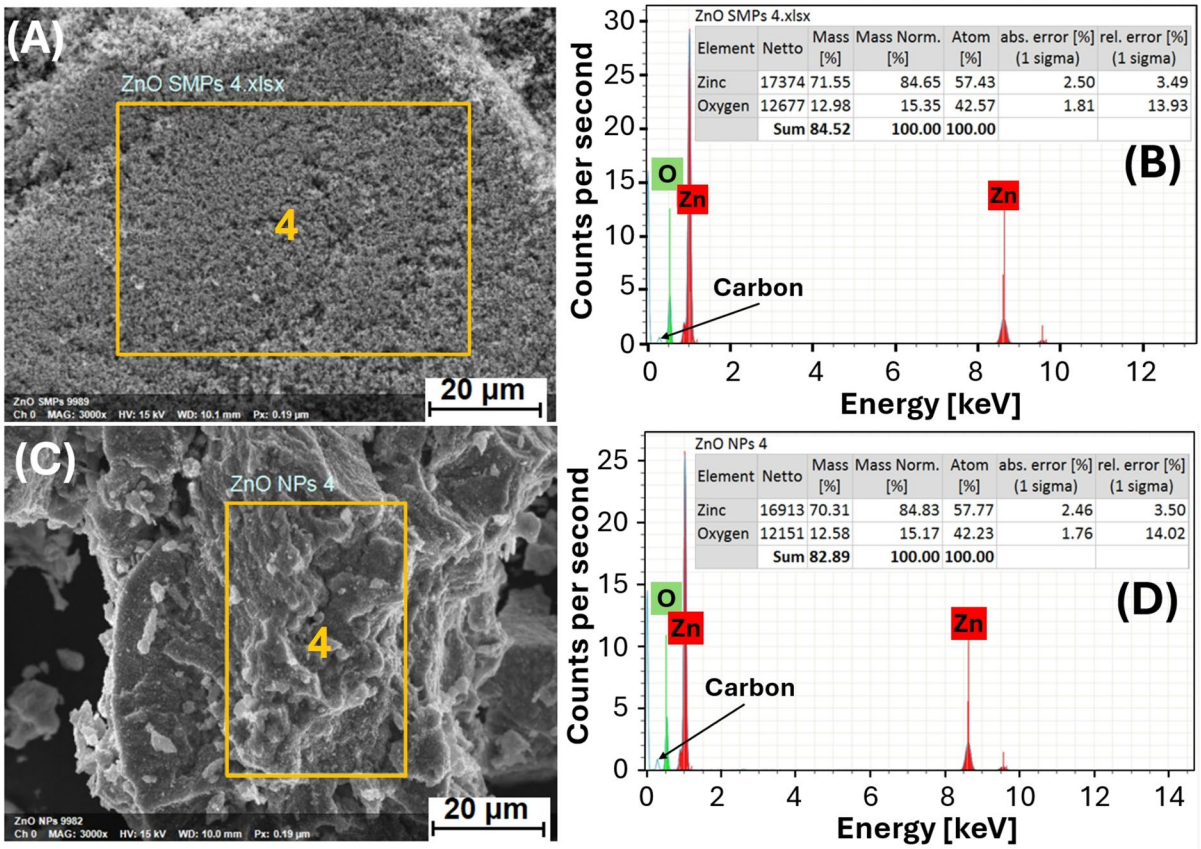

Fig. 4 SEM images of zinc oxide submicron particles (ZnO SMPs) (**A**) and zinc oxide nanoparticles (ZnO NPs) (**C**). The quadrilateral marked with the number 4 indicates the location of the EDS analysis. The representative EDS spectrum of element analysis of sample ZnO SMPs (**B**) and sample ZnO NPs (**D**)

Table 1 Characteristics of zinc oxide samples: zinc oxide nanoparticles (ZnO NPs) and zinc oxide submicron particles (ZnO SMPs) (Reprinted with permission from Tymoszuk & Wojnarowicz, 2020)

Sample name	Skeleton density, $\rho_s \pm \sigma$ (g/m^3)	Specific surface area (SSA), a_s (m^2/g^1)	Average particle size from SSA, d ± σ (nm)	Average crystallite size, Scherrer's formula, d ± σ (nm)
zinc oxide nanoparticles	5.24 ± 0.05	38.8	30 ± 2	27 ± 3
zinc oxide submicron particles	5.59 ± 0.03	4.5	240 ± 30	124 ± 11

A concentration of 500 mg/l was sufficient to limit pathogen growth, and higher concentrations did not provide additional benefits. Regardless of the type of zinc oxide sample, the use of the lowest concentration—i.e. 100 mg/l accelerated the growth of the mycelium, only at a concentration of 500 mg/l and higher the growth of the pathogen was inhibited. Whereas, regardless of the concentration, the type of zinc oxide sample used did not affect the diameter of the mycelium (Table 2, Fig. 6A).

In vivo experiment with tomato plants showed that, regardless of the date of evaluation, the use of ZnO SMPs did not reduce the spread of *B. cinerea* infection as compared to infected control plants, unlike ZnO NPs. The use of ZnO NPs resulted in the significant reduction in the pathogen infection

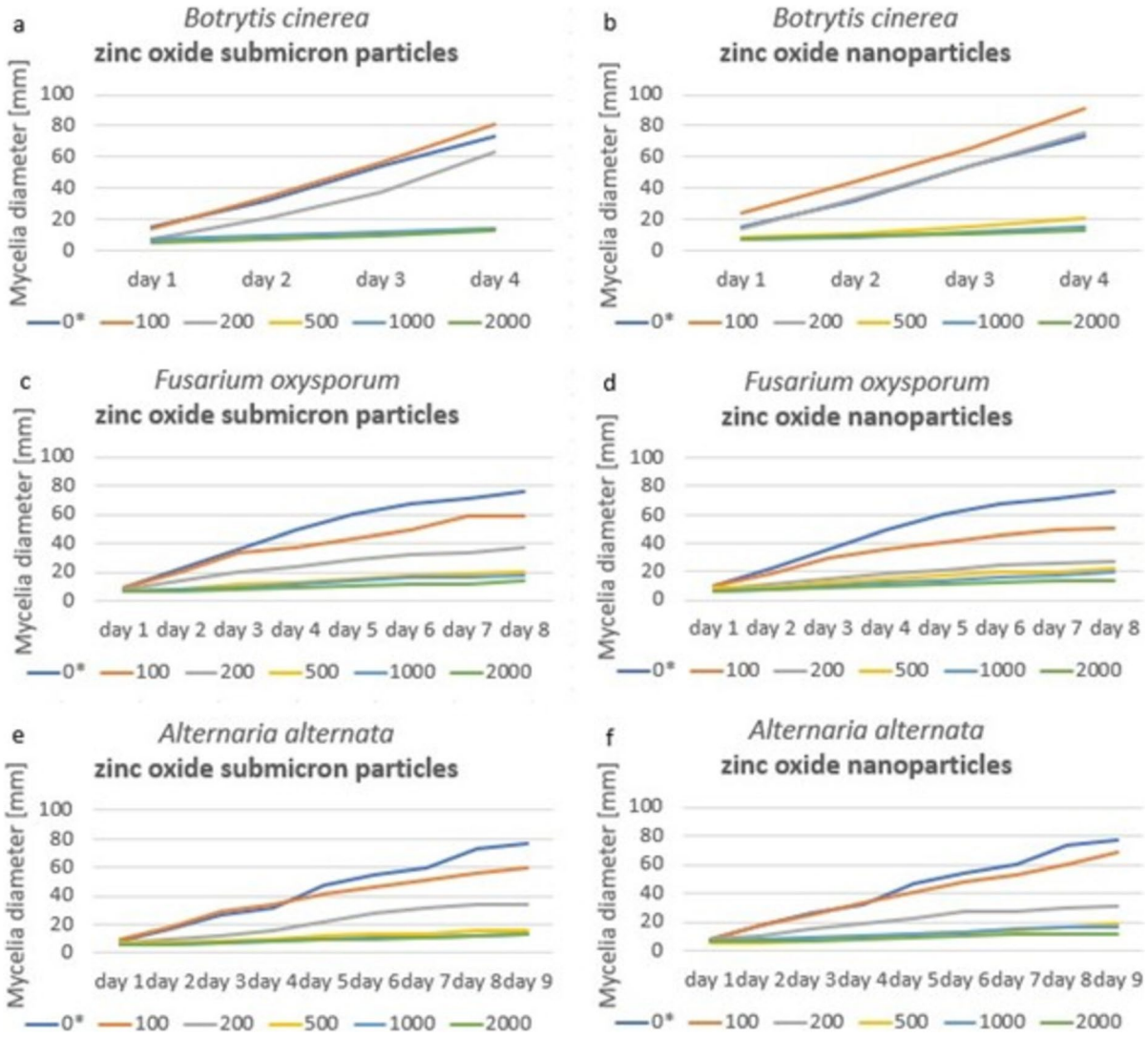

Fig. 5 Mycelium growth dynamics (in days) of *B. cinerea* (**a**-**b**), ***F***. *oxysporum* (**c**-**d**), and ***A***. *alternata* (**e**–**f**) as a function of zinc oxide sample type and concentration (*mg/l)

(57.1%). High antifungal activity of ZnO NPs against *B. cinerea* was confirmed in both evaluation dates (Table 3, Fig. 7A-B).

Effects of zinc oxide submicron particles and zinc oxide nanoparticles on the inhibition of *F. oxysporum*

Pathogen completely overgrown the Petri plate in eight days in control, twice as long as in *B. cinerea*. When ZnO SMPs were used, the growth rate was the highest at the beginning of the observation, in the control in the first four days—the pathogen grew, on average, 12.80 mm per day. The application of ZnO SMPs at higher concentrations resulted in lower growth dynamics. A similar dependence was observed for ZnO NPs (Fig. 5C-D). Both ZnO SMPs and ZnO NPs significantly inhibited the growth of *F. oxysporum* mycelium already at the lowest tested concentration (100 mg/l). ZnO NPs limited the growth of mycelium to a greater extent than ZnO SMPs only at concentrations of 100 mg/l and 200 mg/l. The statistically similar inhibitory effect was obtained at higher tested concentrations, with the greatest inhibition of mycelium growth observed at the highest tested

Table 2 Mycelial diameter (mm) of *B. cinerea* depending on the zinc oxide sample type and concentration

Sample concentration (mg/l)	Sample type		Mean
	zinc oxide submicron particles	zinc oxide nanoparticles	
0	72.70 ± 13.67a*	72.70 ± 13.67a	72.70B
100	81.60 ± 0.89a	81.63 ± 0.96a	81.62A
200	63.30 ± 8.05b	75.40 ± 13.40a	69.35B
500	13.90 ± 0.65c	20.50 ± 4.69c	17.20C
1000	13.70 ± 1.25c	15.30 ± 0.57c	14.50C
2000	12.60 ± 1.24c	12.70 ± 1.35c	12.65C
Mean	42.97A	46.37A	

*Means ± Standard Deviation followed by the same letter do not differ significantly at $p \leq 0.05$ (Fisher test). Upper-case letters refer to the main effects (irrespectively), lower-case letters refer to the interaction between the two studied independent variables

concentration. Regardless of the type of zinc oxide sample, mycelium growth was inhibited on media modified with the lowest concentration—i.e. 100 mg/l, while concentrations of 500 mg/l and 1000 mg/l gave a similar result. The greatest growth reduction was observed for the highest concentration. Regardless of the concentration and the type of zinc oxide sample used did not affect the diameter of the mycelium, similarly to *B. cinerea* (Table 4, Fig. 6B).

In vivo experiment showed that, regardless of the date of the evaluation, the application of ZnO NPs gave a better result in the reduction of *F. oxysporum* infection (65.7%) than ZnO SMPs (44.4%). Although after the first evaluation was noted that the use of ZnO SMPs and ZnO NPs resulted in a similar level of the diseased leaf area, after the second evaluation leaves of plants treated with ZnO NPs were less infected (Table 5, Fig. 7C).

Effects of zinc oxide submicron particles and zinc oxide nanoparticles on the inhibition of *A. alternata*

Pathogen completely overgrown the Petri plate in nine days in control, and its growth was slower as compared to *B. cinerea* and *F. oxysporum*. When ZnO

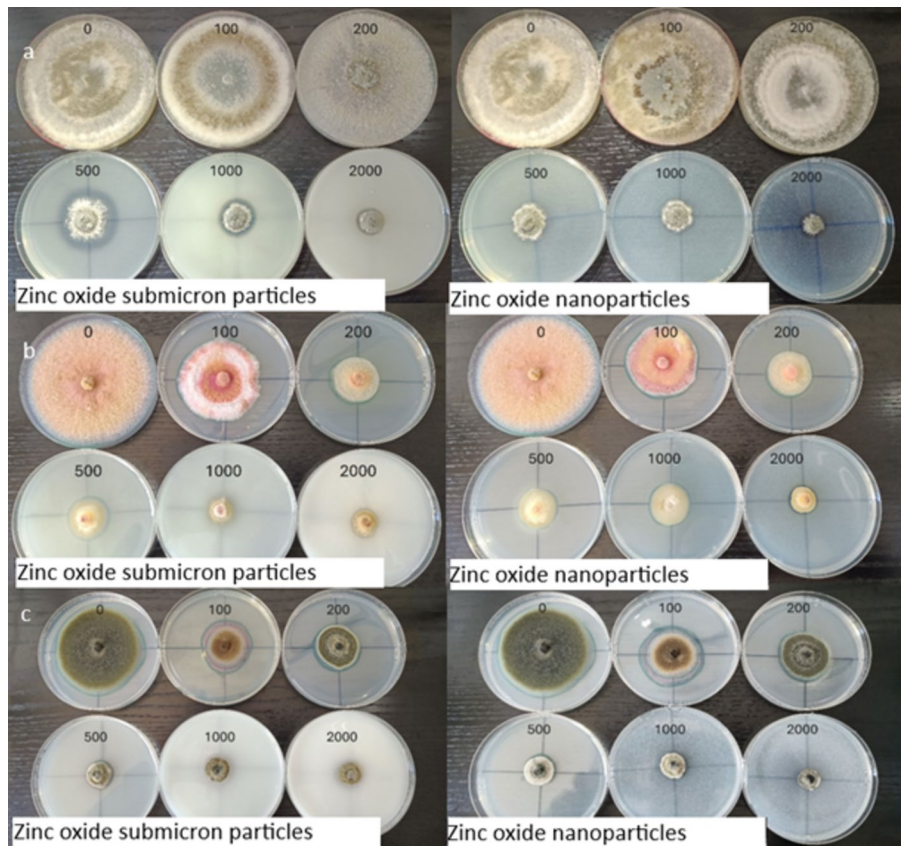

Fig. 6 The effect of zinc oxide sample type and concentration (0–2000 mg/l) on mycelia growth of *B. cinerea* after 4 days (**a**), *F. oxysporum* after 8 days and (**b**), and *A. alternata* after 9 days (**c**)

Table 3 Diseased leaf area (%) of *S. lycopersicum* cv. 'Bawole Serce' plants infected with *B. cinerea*, depending on the treatment and evaluation date

Treatment	Evaluation		Mean
	1st	2nd	
Non-infected control plants	0.00 ± 0.00e*	0.00 ± 0.00e	0.00C
Infected control plants	6.35 ± 1.19bc	17.32 ± 1.75a	11.84A
Infected and 500 mg/l zinc oxide submicron particles sprayed plants	5.84 ± 1.00c	16.61 ± 1.84a	11.23A
Infected and 500 mg/l zinc oxide nanoparticles sprayed plants	2.71 ± 0.83d	7.45 ± 2.48b	5.08B
Mean	3.73B	10.35A	

*Means ± Standard Deviation followed by the same letter do not differ significantly at $p \leq 0.05$ (Fisher test). Upper-case letters refer to the main effects (irrespectively), lower-case letters refer to the interaction between the two studied independent variables

SMPs and ZnO NPs were used at the lowest tested concentration (100 mg/l), the pathogen grew the fastest in the first days. A slower growth dynamic was observed from the fifth day (except for the last day of observation for ZnO NPs), and at higher concentrations the tendency was not entirely clear (when using a concentration of 200 mg/l, the pathogen grew the fastest between the fourth and sixth day, with higher concentrations at the end of the observation period) (Fig. 5E-5F). ZnO SMPs limited *A. alternata* mycelium growth at the lowest concentration, while ZnO NPs showed an inhibitory effect at 200 mg/l. The greatest inhibition of pathogen growth was noted at the concentration of 500 mg/l and above, with statistically similar results for both ZnO forms. Regardless of the type of zinc oxide sample, the growth of the pathogen was limited even when the lowest concentration of samples was used (100 mg/l), while the use of concentrations from 500 mg/l to 2000 mg/l gave a similar effect. Regardless of the concentration, the type of zinc oxidel sample used did not affect the diameter of the mycelium, similarly to *B. cinerea* and *F. oxysporum* (Table 6, Fig. 6C).

Experiment with tomato plants showed that, regardless of the date of the evaluation, the use of ZnO SMPs did not reduce the *A. alternata* infection, unlike ZnO NPs (reduction of 54,8%). Both, in the first and second evaluations, the plants treated with ZnO NPs were significantly less infected, which was not observed for ZnO SMPs (Table 7, Fig. 7D).

Discussion

Zinc and Zn-based NPs have the potential to be exploited as control agents against plant fungal diseases. Our study includes preliminary *in vitro* and *in vivo* tests to explore these effects further in *S. lycopersicum*, one of the most important vegetable crops. The world's tomato cultivation area has increased by 164% in recent decades, and consumption has increased by over 300%, which proves the continued popularity of this vegetable in the global human diet (Nicola et al., 2009).

In this study, the type of zinc oxide sample (ZnO SMPs or ZnO NPs) demonstrated statistically similar effects on *in vitro* mycelial growth across all tested pathogens, regardless of concentration. Both ZnO SMPs and ZnO NPs were equally effective in pathogen growth inhibition. This is consistent with findings from previous research, which showed that different zinc particle sizes affect the initial zinc solubility but do not significantly alter the final concentration of zinc ions in the medium or their biological effects (Tymoszuk & Wojnarowicz, 2020). However, significant statistical interactions between the material type and concentration were observed for *B. cinerea*, *F. oxysporum*, and *A. alternata*. For *B. cinerea*, ZnO SMPs at a concentration of 200 mg/l, showed higher pathogen inhibition as compared to ZnO NPs. Similarly, for *A. alternata*, a concentration of 100 mg/l of ZnO SMPs was more effective than ZnO NPs. On the other hand, for *F. oxysporum*, ZnO NPs exhibited superior inhibitory effects at concentrations of 100 mg/l and 200 mg/l as compared to ZnO SMPs. These findings suggest a pathogen-specific response to zinc nanoparticle application. Moreover, the toxicity of ZnO NPs to microorganisms appears to depend on several factors, including the material concentration, the type of microorganism, and the exposure time.

Ilkhechi et al. (2021) observed that mycelia treated with ZnO-TiO_2 nanostructures had deformed conidia of abnormal shapes and sizes, fragmented cell surfaces, irregular septa caused by cell wall lysis, cytoplasmic disruption, and damaged

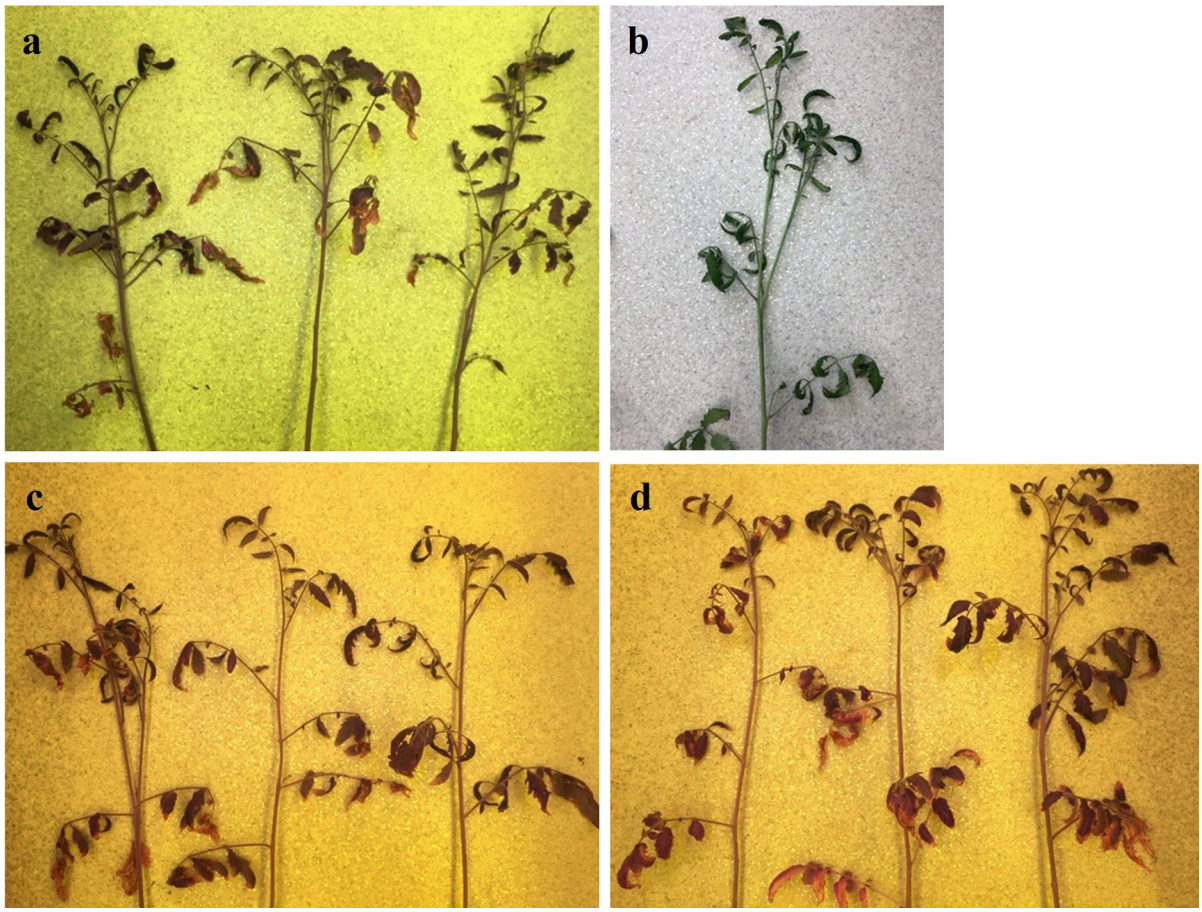

Fig. 7 *S. lycopersicum* 'Bawole Serce' plants infected with *B. cinerea* (**a**), from the left: infected control plant; infected and 500 mg/l zinc oxide submicron particles sprayed plant; infected and 500 mg/l zinc oxide nanoparticles sprayed plant; an example of non-infected, control plant (**b**); *S. lycopersicum* cv. Bawole Serce' plants infected with *F. oxysporum* (**c**), from the left: infected control plant; infected and 500 mg/l zinc oxide submicron particles sprayed plant; infected and 500 mg/l zinc oxide nanoparticles sprayed plant; *S. lycopersicum* cv.'Bawole Serce' plants infected with *A. alternata* (**d**), from the left: infected control plant; infected and 500 mg/l zinc oxide submicron particles sprayed plant; infected and 500 mg/l zinc oxide nanoparticles sprayed plant; at the end of the *in vivo* experiment

mitochondria and the nucleus. ZnO NPs also caused morphological abnormalities in *Fusarium equiseti* isolated from infected tomato plants. According to the authors, the antifungal effect is due to direct or electrostatic contact of nanoparticles with the cell membrane, which disrupts membrane permeability and induces oxidative stress that inhibits cell development (Subba et al., 2024). Dynamic physicochemical interactions, kinetic and thermodynamic exchanges, and van der Waals forces occur between the surface of nanoparticles and the surface of biological components such as membranes and proteins. All these interactions are believed to affect fungal cells, leading to the accumulation of nanoparticles at the cell membrane and consequently to membrane damage. Additionally, an important factor of cytotoxicity is the adhesion of nanoparticles to fungus cells (Djearamane et al., 2022). Simultaneously, Zn particles enhance plant cellular integrity and stimulate the activity of antioxidant enzymes, such as superoxide dismutase (SOD), and defense-related proteins (Jalil et al., 2023).

Dimkpa et al. (2013) added ZnO NPs suspension into PDA medium, similarly to the methodology used in our research. The ZnO NPs showed significantly higher inhibitory effects on *Fusarium graminearum*

Table 4 Mycelia diameter (mm) of *F. oxysporum*, depending on the zinc oxide sample type and concentration

Sample concentration (mg/l)	Sample type		Mean
	zinc oxide submicron particles	zinc oxide nanoparticles	
0	76.30 ± 4.78a*	76.30 ± 4.78a	76.30A
100	58.37 ± 4.87b	50.87 ± 10.38c	54.62B
200	36.80 ± 10.48d	27.50 ± 1.97e	32.15C
500	20.60 ± 2.95 fg	21.90 ± 1.88ef	21.25D
1000	18.30 ± 2.08fgh	19.30 ± 3.17fgh	18.80D
2000	14.10 ± 0.65gh	13.90 ± 1.71 h	14.00E
Mean	37.41A	34.96A	

*Means ± Standard Deviation followed by the same letter do not differ significantly at $p \leq 0.05$ (Fisher test). Upper-case letters refer to the main effects (irrespectively), lower-case letters refer to the interaction between the two studied independent variables

Table 5 Diseased leaf area (%) of *S. lycopersicum* cv. 'Bawole Serce' plants infected with *F. oxysporum*, depending on the treatment and evaluation date

Treatment	Evaluation		Mean
	1st	2nd	
Non-infected control plants	0.00 ± 0.00e*	0.00 ± 0.00e	0.00D
Infected control plants	4.95 ± 0.65c	13.73 ± 1.54a	9.34A
Infected and 500 mg/l zinc oxide submicron particles sprayed plants	2.15 ± 0.97d	8.23 ± 2.16b	5.19B
Infected and 500 mg/l zinc oxide nanoparticles sprayed plants	1.61 ± 0.57d	4.79 ± 1.34c	3.20C
Mean	2.18B	6.69A	

*Means ± Standard Deviation followed by the same letter do not differ significantly at $p \leq 0.05$ (Fisher test). Upper-case letters refer to the main effects (irrespectively), lower-case letters refer to the interaction between the two studied independent variables

Table 6 Mycelial diameter (mm) of *A. alternata*, depending on the zinc oxide sample type and concentration

Sample concentration (mg/l)	Sample type		Mean
	zinc oxide submicron particles	zinc oxide nanoparticles	
0	76.60 ± 8.69a*	76.60 ± 8.69a	76.60A
100	59.30 ± 12.69b	69.17 ± 14.49a	64.24B
200	34.40 ± 3.17c	31.75 ± 5.76c	33.08C
500	16.20 ± 1.15d	19.50 ± 1.00d	17.85D
1000	13.90 ± 1.14d	17.20 ± 2.33d	15.55D
2000	13.30 ± 1.04d	12.20 ± 0.84d	12.75D
Mean	35.62A	37.74A	

*Means ± Standard Deviation by the same letter do not differ significantly at $p \leq 0.05$ (Fisher test). Upper-case letters refer to the main effects (irrespectively), lower-case letters refer to the interaction between the two studied independent variables

Table 7 Diseased leaf area (%) of *S. lycopersicum* cv. 'Bawole Serce' plants infected with *A. alternata*, depending on the treatment and evaluation date

Treatment	Evaluation		Mean
	1st	2nd	
Non-infected control plants	0.00 ± 0.00d*	0.00 ± 0.00d	0.00C
Infected control plants	6.82 ± 0.86b	15.68 ± 2.22a	11.25A
Infected and 500 mg/l zinc oxide submicron particles sprayed plants	5.95 ± 1.00b	16.21 ± 2.90a	11.08A
Infected and 500 mg/l zinc oxide nanoparticles sprayed plants	3.23 ± 1.21c	6.93 ± 1.44b	5.08B
Mean	4.00B	9.71A	

*Means ± Standard Deviation followed by the same letter do not differ significantly at $p \leq 0.05$ (Fisher test). Upper-case letters refer to the main effects (irrespectively), lower-case letters refer to the interaction between the two studied independent variables

growth as compared to Zn microparticles in all tested concentrations (0, 10, 50, 250, 1000 mg/l). Notably, fungal inoculum transferred to a fresh medium after exposure to ZnO NPs did not demonstrate adaptation to the stress, as growth remained inhibited. Similarly, Pan et al. (2022) highlighted the potential of ZnO NPs as green chemical agents to overcome apple replant disease (ARD) and other crop diseases. Treatment with ZnO NPs reduced the prevalence of *Neocosmospora*, *Gibberella*, and *Fusarium* while increasing populations of *Tausonia*, *Chaetomium*, and *Mrakia*. Specifically, the colony-forming units (CFU) of *Fusarium solani* and *F. oxysporum* were reduced by 55.7% and 68.9%, respectively, in the ZnO NPs treatment combination as compared to controls. Application of ZnO NPs to soil also promoted the development of new

microbial community structures conducive to plant growth, aiding in ARD mitigation.

In the present study, pathogen growth inhibition was observed starting from day 1, particularly at concentrations of 200 mg/l and above, and this effect persisted throughout the whole observation period. The most substantial inhibition of *F. oxysporum* growth occurred at the highest concentrations tested, with a statistically significant difference for 200 mg/l treatment. On day 8, the inhibition rate reached 63.96%. Comparable results were reported by Pan et al. (2022), where growth inhibition was 63% after 7 days at 250 mg/l. A similar trend was observed in *A. alternata* in the current study, while for *B. cinerea*, only concentrations of 500 mg/l and higher were most effective.

ZnO NPs significantly inhibited *F. oxysporum* mycelial growth even at the lowest concentration tested (100 mg/l). Notably, ZnO NPs exhibited greater inhibitory effects as compared to ZnO SMPs at concentrations of 100 mg/l and 200 mg/l. A similar effect was observed at 500 mg/l, with the strongest mycelial growth inhibition achieved at the highest concentrations, 1000 mg/l and 2000 mg/l. The enhanced antifungal performance of ZnO NPs can be assigned to optimized particle parameters, such as crystalline structure, size, concentration, and preparation methods. Imran et al. (2023) reported that foliar application of ZnO NPs (10 mg/l) on tomato plants under greenhouse conditions reduced *B. cinerea* symptoms by 84.1%, decreasing disease severity from 82.18% in the untreated control to 12.81%. Similarly, Tryfon et al. (2023) found that ZnO NPs showed high antifungal activity against *B. cinerea*, reducing disease index to levels observed for traditional fungicides (e.g., Luna SC) in tomato and cucumber pot trials.

The effectiveness of ZnO NPs in managing early blight, caused by *A. solani*, has been demonstrated in previous studies. For three tomato cultivars highly susceptible to the disease, foliar application of ZnO NPs at concentrations of 3 and 5 g/l reduced disease severity to 17.04% and 20.13% respectively, as compared to 30.6% in the untreated control. Notably, the ZnO NPs treatment outperformed the fungicide Beltanol, which reduced symptoms to 26.18% (Lahuf et al., 2020). Studies conducted so far have shown that the use of chemically and biologically synthesized ZnO NPs at various doses reduced the severity of *A. solani* symptoms on tomatoes by about 20% (Aldayel et al., 2024). ZnO NPs have been reported as promising antifungal against other *Alternaria* species. For instance, Elshafie et al. (2023) demonstrated that post-harvest treatment with ZnO NPs reduced the severity of citrus black rot, caused by *Alternaria citri*, to between 6.92% and 9.23%, as compared to 23.84% in untreated fruits after 20 days of storage. In a field experiment, Mahmoud (2023) found that ZnO NPs applied at a concentration of 20 µg/ml completely inhibited Alternaria leaf spot in beans caused by *A. alternata*, while the control plants exhibited full disease development.

González-Merino et al. (2021) observed that ZnO NPs at concentrations of 1,500–3,000 mg/l effectively limited the spread of *F. oxysporum* in tomato plants under greenhouse conditions. Notably, in their experiment, ZnO particles (non-nanoscale) were less effective than ZnO NPs, which is consistent with the findings presented in this study. ZnO NPs have demonstrated efficacy against *F. oxysporum* in other crops as well. For instance, they suppressed *F. oxysporum* in pepper plants by 81.81% (Abdelaziz et al., 2021).

In the present study, foliar application of ZnO NPs proved to be effective in managing symptoms caused by *B. cinerea, F. oxysporum*, and *A. alternata*. Symptoms of all tested pathogens were reduced by over 50%, with ZnO NPs being particularly effective against *F. oxysporum.* On average, symptoms caused by *F. oxysporum* were reduced by 65.7%—from 4.95% of the infected leaf area to 1.61% on the first observation date and from 13.73% to 4.79% on the second observation date. While ZnO SMPs significantly inhibited the *in vitro* mycelial growth of all three pathogens, their efficacy *in vivo* was limited. Specifically, ZnO SMPs were ineffective against *B. cinerea* and *A. alternata* and less effective than ZnO NPs against *F. oxysporum.* Therefore, it should be borne in mind that the effects of the same zinc oxide samples on fungal pathogens may differ significantly in the environment of axenic *in vitro* culture compared to *in vivo* conditions.

In a study conducted by Pejam et al. (2021), ZnO NPs were more effective than ZnO SMPs in improving growth, yield and metabolism, and modifying transcriptional programs in tomato. However, the mechanism by which ZnO NPs can impart partially differentiated responses remains controversial. Most researchers believe that the diverse physicochemical properties of nanoparticles contributed to the

differences in their uptake kinetics, subsequent interactions with biomolecules, signalling, and responses. According to our observations, submicron particles were more phytotoxic to tomato plants. After application, ZnO SMPs formed a mechanical layer on the tissue surface, in contrast to smaller ZnO NPs.

Nanoparticles can enter the plant through roots, leaves or stems, and can be transferred to other parts through the xylem and phloem, even to fruits and seeds. Nanoparticles, by modulating physiology and photosynthetic activity, can increase growth rate and stress tolerance in plants. In addition, the use of nanoparticles can improve the nutritional status of plants, leading to plant products with greater nutritional value for human health (Haq et al., 2024). Sharifan et al. (2022) proved that spraying tomato fruits with zinc oxide nanoparticles after harvest improved durability and increased the concentration of zinc in the fruit, and was also associated with a 47% inhibition of microbial growth. The positive effects of Zn and ZnO NPs on tomato production also include improved plant health, better nutritional quality of fruits, increased fruit quantity per plant, and higher yields (Ahmed et al., 2021). The benefits of nanotechnology in agriculture are worthy of attention, both in terms of phytopathology and plant yielding and quality.

The presented research results concern the method of limiting the growth of mycelium of plant pathogens of the species *A. alternata, B. cinerea and F. oxysporum* using zinc oxide submicron particles or zinc oxide nanoparticles characterized by strictly defined physicochemical parameters, which, at an appropriate concentration, reduced the growth of pathogens by more than half. However, Kairyte et al. (2013) emphasize the need for further research to better understand the mechanisms responsible for the antimicrobial action of ZnO NPs, as well as their potential genotoxic effects. Malandrakis et al. (2022) highlight concerns regarding the unintended effect of ZnO NPs on non-target organisms, such as potential phytotoxicity, human toxicity, and environmental ecotoxicity. These risks must be thoroughly evaluated before the large-scale commercialization of ZnO NPs as nanopesticides. Furthermore, there is insufficient evidence to definitively conclude that nano-sized particles pose greater hazards than their larger counterparts, such as microparticles or bulk materials. Aldayel et al. (2024) also point out the need for deeper investigation into critical aspects of nanoscale ZnO, including its penetration, migration, accumulation, and biodegradation. Such studies are essential to accurately assess the biosafety of ZnO NPs and ensure their responsible application in agricultural practices. Zinc is an indispensable element in the metabolic processes of humans and animals. Its daily intake for an adult is 8–15 mg (Siddiqi et al., 2018). Studies suggest that ZnO NPs can persist in plant tissues and be transferred to higher trophic levels via food consumption, posing a risk if they accumulate to toxic levels (Haq et al., 2024). Zinc oxide is known to be safe for humans and is commonly used in cosmetics and dietary supplements. Various biomedical applications of ZnO NPs are currently being investigated (El-Saadony et al., 2024). Therefore, the topic remains open and worthy of attention, both in terms of selecting particles with the most favorable parameters and determining their influence on other aspects mentioned above.

Conclusions

ZnO SMPs and ZnO NPs characterized by strictly defined physicochemical parameters significantly inhibited *in vitro* mycelium growth in *B. cinerea*, *F. oxysporum*, and *A. alternata*.

As for *B. cinerea* and *A. alternata*, the use of ZnO SMPs or ZnO NPs at the concentration of 500 mg/l provided a similarly high inhibitory effect as the use of these samples at higher tested concentrations. In *F. oxysporum*, mycelium diameter decreased with increasing ZnO concentration, and the highest inhibition was observed at 2000 mg/l, regardless of the type of ZnO sample used. *In vivo* experiments verifying the results observed *in vitro* and assessing the effectiveness of ZnO SMPs and ZnO NPs as potential fungicides in plant protection did not confirm the effectiveness of ZnO SMPs in limiting the growth of *B. cinerea* and *A. alternata*.

The use of ZnO NPs significantly reduced the infection of all tested pathogens: *B. cinerea* (57.1%), *F. oxysporum* (65.7%) and *A. alternata* (54.8%).

Although our study provides insight into the antifungal activity of ZnO NPs and SMPs, some limitations must be acknowledged. Firstly, the study was conducted under *in vitro* and *in vivo* controlled conditions, which may not fully reflect the complexity of field environments, where factors such as soil

composition, microbial interactions and environmental stress can affect particle efficacy. Additionally, the long-term effects of ZnO NPs on plant health, soil microbiota and potential phytotoxicity remain unexplored. Future research needs to be conducted to assess the applicability of ZnO NPs in the field, optimise dosage and application methods, and evaluate their environmental impact over longer periods. Investigating synergistic effects with other biocontrol agents and developing environmentally friendly formulations to minimise potential toxicity may also further our understanding of their use as control agents.

Patents

The elaborated method was sent to the Patent Office of the Republic of Poland as a patent application no. P.445676: "Method of limiting the growth of mycelium of plant pathogens of the species *Alternaria alternata*, *Botrytis cinerea* and *Fusarium oxysporum* using zinc oxide or zinc oxide nanoparticles" (Polish title: "Sposób ograniczania wzrostu grzybni patogenów roślinnych z gatunków *Alternaria alternata*, *Botrytis cinerea* oraz *Fusarium oxysporum* z zastosowaniem tlenku cynku lub nanocząstek tlenku cynku").

Acknowledgements The Authors would like to thank Prof. Dariusz Kulus for his professional support in manuscript correction and Jan Mizeracki for EDS analysis. Sample characterisation studies were conducted on equipment funded by the project Center for Preclinical Research and Technology—CePT I (POIG.02.02.00-14-024/08), financed by the European Regional Development Fund "Innovative Economy" for years 2007–2013 and CePT II (RPMA.01.01.00- 14-8476/17-04) from Regional Operational Programme of the Mazowieckie Voivodeship 2014–2020".

Author contributions All authors made a significant contribution to the work presented. All authors have read and agreed to the published version of the manuscript.

Funding This research received no external funding.

Data availability Data available by e-mail on reasonable request.

Declarations

Institutional review board statement Not applicable.

Conflicts of interest The authors declare no conflict of interest.

References

Abdelaziz, A. M., Dacrory, S., Hashem, A. H., Attia, M. S., Hasanin, M., Fouda, H. M., Kamel, S., & ElSaied, H. (2021). Protective role of zinc oxide nanoparticles based hydrogel against wilt disease of pepper plant. *Biocatalysis and Agricultural Biotechnology, 35*, Article 102083. https://doi.org/10.1016/j.bcab.2021.102083

Ahmed, R., YusoffAbdSamad, M., Uddin, M. K., Quddus, M. A., & Hossain, M. M. (2021). Recent trends in the foliar spraying of zinc nutrient and zinc oxide nanoparticles in tomato production. *Agronomy, 11*(10), 2074. https://doi.org/10.3390/agronomy11102074

Aldayel, M. F., Alrajeh, H. S., Sallam, N. M. A., & Imran, M. (2024). *Bacillus amyloliquefaciens* IKMM and zinc nanoparticles as biocontrol candidate induce the systemic resistance by producing antioxidants in tomato plants challenged with early blight pathogen. *Journal of Crop Health, 76*(1), 87–103. https://doi.org/10.1007/s10343-023-00942-0

Dimkpa, C. O., McLean, J. E., Britt, D. W., & Anderson, A. J. (2013). Antifungal activity of ZnO nanoparticles and their interactive effect with a biocontrol bacterium on growth antagonism of the plant pathogen *Fusarium graminearum*. *BioMetals, 26*(6), 913–924. https://doi.org/10.1007/s10534-013-9667-6

Djearamane, S., Xiu, L.-J., Wong, L.-S., Rajamani, R., Bharathi, D., Kayarohanam, S., De Cruz, A. E., Tey, L.-H., Janakiraman, A. K., Aminuzzaman, M., & Selvaraj, S. (2022). Antifungal properties of zinc oxide nanoparticles on *Candida albicans*. *Coatings, 12*(12), 1864. https://doi.org/10.3390/coatings12121864

El-Saadony, M. T., Saad, A. M., Soliman, S. M., Salem, H. M., Ahmed, A. I., Mahmood, M., El-Tahan, A. M., Ebrahim, A. A. M., Abd El-Mageed, T. A., Negm, S. H., Selim, S., Babalghith, A. O., Elrys, A. S., El-Tarabily, K. A., & Abu-Qamar, S. F. (2022). Plant growth-promoting microorganisms as biocontrol agents of plant diseases: Mechanisms, challenges and future perspectives. *Frontiers in Plant*

Science, 13, Article 923880. https://doi.org/10.3389/fpls.2022.923880

El-Saadony, M. T., Fang, G., Yan, S., Alkafaas, S. S., El Nasharty, M. A., Khedr, S. A., Hussien, A. M., Ghosh, S., Dladla, M., Elkafas, S. S., Ibrahim, E. H., Salem, H. M., Mosa, W. F., Ahmed, A. E., Mohammed, D. M., Korma, S. A., El-Tarabily, M. K., Saad, A. M., El-Tarabily, K. A., & AbuQamar, S. F. (2024). Green synthesis of zinc oxide nanoparticles: Preparation, characterization, and biomedical applications - A review. *International Journal of Nanomedicine, 19*, 12889–12937. https://doi.org/10.2147/IJN.S487188

Elshafie, H. S., Osman, A., El-Saber, M. M., Camele, I., & Abbas, E. (2023). Antifungal activity of green and chemically synthesized ZnO nanoparticles against *Alternaria citri*, the causal agent citrus black rot. *The Plant Pathology Journal, 39*(3), 265. https://doi.org/10.5423/PPJ.OA.02.2023.0035

González-Merino, A. M., Hernández-Juárez, A., Betancourt-Galindo, R., Ochoa-Fuentes, Y. M., Valdez-Aguilar, L. A., & Limón-Corona, M. L. (2021). Antifungal activity of zinc oxide nanoparticles in *Fusarium oxysporum-Solanum lycopersicum* pathosystem under controlled conditions. *Journal of Phytopathology, 169*(9), 533–544. https://doi.org/10.1111/jph.13023

Haq, I. U., Cai, X., Ali, H., Akhtar, M. R., Ghafar, M. A., Hyder, M., & Hou, Y. (2024). Interactions between nanoparticles and tomato plants: Influencing host physiology and the tomato leafminer's molecular response. *Nanomaterials (Basel), 14*(22), 1788. https://doi.org/10.3390/nano14221788

Ilkhechi, N., Mozammel, M., & Khosroushahi, A. (2021). Antifungal effects of ZnO, TiO2 and ZnO-TiO2 nanostructures on *Aspergillus flavus*. *Pesticide Biochemistry and Physiology, 176*, Article 104869. https://doi.org/10.1016/j.pestbp.2021.104869

Imran, M., Abo-Elyousr, K. A., El-Sharnouby, M. E., Ali, E. F., Sallam, N. M., Bagy, H. M. K., & Abdel-Rahim, I. R. (2023). Biocontrol potential of *Trichoderma harzianum* and zinc nanoparticles to mitigate gray mold disease of tomato. *Gesunde Pflanzen, 75*(1), 151–163. https://doi.org/10.1007/s10343-022-00686-3

Jalil, S., Alghanem, S. M., Al-Huqail, A. A., Nazir, M. M., Zulfiqar, F., Ahmed, T., Ali, S., Abeed, A. H. A., Siddique, K. H. M., & Jin, X. (2023). Zinc oxide nanoparticles mitigated the arsenic induced oxidative stress through modulation of physio-biochemical aspects and nutritional ions homeostasis in rice (*Oryza sativa* L.). *Chemosphere, 338*, 139566. https://doi.org/10.1016/j.chemosphere.2023.139566

Kairyte, K., Kadys, A., & Luksiene, Z. (2013). Antibacterial and antifungal activity of photoactivated ZnO nanoparticles in suspension. *Journal of Photochemistry and Photobiology, b: Biology, 128*, 78–84. https://doi.org/10.1016/j.jphotobiol.2013.07.017

Kowalska, J., Antkowiak, M., Krzymińska, J., Tymoszuk, A., & Osial, M. (2025). Effects of copper oxide micro- and nanoparticles on *Alternaria alternata*, *Botrytis cinerea* and *Fusarium oxysporum* mycelium growth. *Progress in Plant Protection, 65*(3), 2025. https://doi.org/10.14199/ppp-2025-015

Lahuf, A. A., Abdullah, K. M., & Mohammadali, M. T. (2020). Assessment of the nanosized particles of ZnO and MgO and some cultivars in control of *Alternaria solani* causing tomato early blight. *Ecology Environment and Conservation, 26*(1), 89–95.

Mahmoud, R. K. (2023). Effect of spraying nano zinc on the growth of two cultivars of broad beans infected with alternarial spot. *Euphrates Journal of Agricultural Science, 15*(4), 116–128.

Malandrakis, A., Kavroulakis, N., & Chrysikopoulos, C. V. (2022). Zinc nanoparticles: Mode of action and efficacy against boscalid-resistant *Alternaria alternata* isolates. *Science of the Total Environment, 829*, Article 154638. https://doi.org/10.1016/j.scitotenv.2022.154638

Mgadi, K., Ndaba, B., Roopnarain, A., Rama, H., & Adeleke, R. (2024). Nanoparticle applications in agriculture: Overview and response of plant-associated microorganisms. *Frontiers in Microbiology, 15*, 1354440. https://doi.org/10.3389/fmicb.2024.1354440

Nicola, S., Tibaldi, G., & Fontana, E. (2009). Tomato production systems and their application to the tropics. *Acta Horticulturae, 821*, 27–34. https://doi.org/10.17660/ActaHortic.2009.821.1

Oława H (2024). Chemical composition of pharmaceutical zinc oxide. Available online: https://hutaolawa.pl/en/oferta/zinc-oxide/ (accessed on 13 September 2024).

Pan, L., Zhao, L., Jiang, W., Wang, M., Chen, X., Shen, X., Yin, C., & Mao, Z. (2022). Effect of zinc oxide nanoparticles on the growth of *Malus hupehensis* Rehd. seedlings. *Frontiers in Environmental Science, 10*, Article 835194. https://doi.org/10.3389/fenvs.2022.835194

Pejam, F., Ardebili, Z. O., Ladan-Moghadam, A., & Danaee, E. (2021). Zinc oxide nanoparticles mediated substantial physiological and molecular changes in tomato. *PLoS ONE, 16*(3), Article e0248778. https://doi.org/10.1371/journal.pone.0248778

Sharifan, H., Noori, A., Bagheri, M., & Moore, J. M. (2022). Postharvest spraying of zinc oxide nanoparticles enhances shelf life qualities and zinc concentration of tomato fruits. *Crop and Pasture Science, 73*, 22–31. https://doi.org/10.1071/CP21191

Siddiqi, K. S., Ur Rahman, A., & Tajuddin, H. A. (2018). Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale Research Letters, 13*, 141. https://doi.org/10.1186/s11671-018-2532-3

Subba, B., Rai, G. B., Bhandary, R., Parajuli, P., Thapa, N., Kandel, D. R., Mulmi, S., Shrestha, S., & Malla, S. (2024). Antifungal activity of zinc oxide nanoparticles (ZnO NPs) on *Fusarium equiseti* phytopathogen isolated from tomato plant in Nepal. *Heliyon, 10*(22), Article e40198. https://doi.org/10.1016/j.heliyon.2024.e40198

Tryfon, P., Kamou, N. N., Pavlou, A., Mourdikoudis, S., Menkissoglu-Spiroudi, U., & Dendrinou-Samara, C. (2023).

Nanocapsules of ZnO nanorods and geraniol as a novel mean for the effective control of *Botrytis cinerea* in tomato and cucumber plants. *Plants, 12*(5), 1074. https://doi.org/10.3390/plants12051074

Tymoszuk, A., & Wojnarowicz, J. (2020). Zinc oxide and zinc oxide nanoparticles impact on *in vitro* germination and seedling growth in *Allium cepa* L. *Materials, 13*(12), 2784. https://doi.org/10.3390/ma13122784

Wojnarowicz, J., Chudoba, T., & Lojkowski, W. (2020). A review of microwave synthesis of zinc oxide nanomaterials: Reactants, process parameters and morphologies. *Nanomaterials (Basel), 10*(6), 1086. https://doi.org/10.3390/nano10061086