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Nanoparticles and photodynamic therapy in the treatment and control of *Alternaria alternata* in wheat seeds

Nanopartículas e terapia fotodinâmica no tratamento e no controle de *Alternaria alternata* em sementes de trigo

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ABSTRACT

The expansion of wheat crops (*Triticum* spp.) to the Brazilian Cerrado highlights the need to use pathogen-free seeds. This study aimed at evaluating the efficacy of nanoparticles (NPs) and photodynamic therapy (PhT) in inhibiting the *in vitro* growth of the fungus *Alternaria alternata,* in its treatment and control in naturally contaminated wheat seeds, and in the physiological quality of the seeds. The efficacy of NPs (ZnOCl, ZnOCl:1Cu, ZnOCl:0.1Ag; ZnO:1Cu, ZnO, and ZnO:1Ag) and PhT using methylene blue (MB) and toluidine blue (TB) dyes was evaluated in inhibiting the mycelial growth of *A. alternata* and in the treatment and control of the pathogen in wheat seeds by evaluating germination, emergence, GSI, ESI, accelerated ageing, and health. All NPs at 2.5 and 5 mg mL^{-1} concentrations and the dyes MB, TB, MB + TB at 50 and 100 µmol L^{-1} inhibited mycelial growth and reduced the incidence of *A. alternata* in the seeds. The NP ZnO:1Ag at 5 mg mL^{-1} and the MB + TB dye at 100 µmol L^{-1} were the most effective in inhibiting mycelial growth. NPs and PhT did not affect the physiological quality of seeds and controlled *A. alternata* in wheat seeds, demonstrating potential use in the treatment and control of the pathogen in wheat seeds.

Index terms: Methylene blue; toluidine blue; *Triticum* spp.; ZnO.

RESUMO

Com a expansão da cultura do trigo (*Triticum* spp.) para o Cerrado brasileiro, evidenciando-se a necessidade da utilização de sementes livres de patógenos. O presente trabalho teve como o objetivo avaliar a eficácia de nanopartículas (NPs) e da terapia fotodinâmica (PhT) na inibição do crescimento de *Alternaria alternata in vitro,* no tratamento, no controle do fungo em sementes de trigo naturalmente contaminadas e na qualidade fisiológica das sementes. A eficácia das NPs (ZnOCl, ZnOCl:1Cu, ZnOCl:0,1Ag; ZnO:1Cu, ZnO e ZnO:1Ag) e da PhT com uso dos corantes azul de metileno (MB) e azul de toluidina (TB) foram avaliadas na inibição do crescimento micelial de *A. alternata,* no tratamento, no controle do patógeno em sementes de trigo, avaliando-se a germinação, emergência, IVG, IVE, envelhecimento acelerado e a sanidade das sementes. Todas as NPs nas concentrações 2,5 e 5 mg mL^{-1} e os corantes MB, TB, MB+TB a 50 e 100 µmol L^{-1} inibiram o crescimento micelial e reduziram a incidência de *A. alternata* nas sementes. A NP de ZnO:1Ag a 5 mg mL^{-1} e os corantes MB+TB a 100 µmol L^{-1} foram os mais eficazes na inibição do crescimento micelial. As NPs e PhT não afetaram a qualidade fisiológica das sementes e controlaram *A. alternata* nas sementes de trigo, demonstrando potencial de uso no tratamento e no controle do patógeno em sementes de trigo.

Termos para indexação: Azul de metileno; azul de toluidina; *Triticum* spp.; ZnO.

INTRODUCTION

The expansion of wheat crops (*Triticum* spp.) to the Brazilian Cerrado (Souza; Vieira Filho, 2020) and the occurrence of diseases that can lead to crop losses of up to 60% in southern Brazil (Maciel et al., 2020) evidence the need to use pathogen-free seeds.

Using healthy seeds is one of the main measures to be implemented in the management of diseases, reducing the pathogen spread and introduction to new areas and protecting the seed from eventual infections or productivity reduction (Nascimento et al., 2021).

In search of viable and safe alternatives for the treatment of seeds, studies on nanoparticles (NPs) and photodynamic therapy (PhT) have gained relevance as these methods have great potential in controlling plant diseases (Shang et al., 2019; Zancan; Tebaldi, 2020; Fraga et al., 2021; Ferreira; Tebaldi; Oliveira, 2021; Mamede et al., 2022).

NPs have a reduced size (less than 100 nm), high reactivity (Kah; Hofmann, 2014) and high biocidal efficacy, interacting with the microbial membrane due to a high surface/volume ratio (Allaker, 2010), being useful in the control of pathogenic microorganisms as they rarely generate resistance, allowing a greater product longevity (Botelho et al., 2020).

PhT is a procedure used in the medical field to treat skin diseases with photoreactive dyes such as methylene blue (MB) and toluidine blue (TB) (Issa; Manela-Azulay, 2010). It has the advantage of presenting a low risk of toxicity (Kübler, 2005) and the ability to inactivate a wide range of microorganisms, since the dyes produce reactive oxygen species (ROS) when irradiated, disintegrating the pathogen membrane and DNA (Perussi, 2007).

As for plant disease controls, the ZnO:0.5Mo, ZnO:1K, and ZnO:1Mg NPs reduced the severity of the bacterial spot of tomato and the presence of *Xanthomonas gardneri* in tomato seeds (Fraga et al., 2021). The severity of maize white spot caused by *Pantoea ananatis* reduced with the use of ZnO:0.1Cu and ZnO:0.2Mn NPs (Mamede et al., 2022). In PhT, the MB and TB dyes inhibited the growth of *X. campestris* pv. *campestris* (Zancan; Tebaldi, 2020) and reduced the presence of *X. gardneri* in tomato seeds (Ferreira; Tebaldi; Oliveira, 2021).

However, *Alternaria alternata*, which causes black point in wheat seeds, is a cosmopolitan fungus that can infect several plant species and cause losses that make productive capacity unfeasible (Xiao; Bergeron; Lau, 2012). Wheat seeds contaminated with this pathogen are the main source of inoculum, as the fungus maintains its viability in the off-season during storage (Casa et al., 2012). Faced with the challenge of finding alternatives for controlling *A. alternata* in wheat seeds, this study aimed at evaluating the effectiveness of NPs and PhT with MB and TB dyes in the growth inhibition of *A. alternata in vitro,* in the treatment and control of the pathogen in naturally contaminated wheat seeds, and in the physiological quality of the seeds.

MATERIAL AND METHODS

The experiments were conducted at the Laboratório de Bacteriologia Vegetal and Laboratório de Sementes, of the Instituto de Ciências Agrárias, Universidade Federal de Uberlândia (UFU), MG, Brazil. Wheat seeds of the cultivar TBio Toruk were produced in the 2019 crop, being harvested in October in the experimental area of Universidade Estadual Paulista (UNESP), Dracena, SP, Brazil. The ZnO, ZnO:1Ag, ZnO:1Cu, ZnOCl, ZnOCl:0.1Ag, and ZnOCl:1Cu NPs were synthesized at the Laboratory of New Insulating and Semiconductor Materials, Instituto de Física, UFU, according to the method described by Silva et al. (2018).

Evaluation of the initial sanitary quality of wheat seeds

The initial sanitary quality of the wheat seeds was evaluated using the filter paper test (Brasil, 2009). The seeds were placed on two sheets of sterile blotting paper moistened with distilled water at a proportion of 2.5-fold the weight of the paper in gerbox (12 x 12 x 5 cm). A total of 200 wheat seeds were used in four replications of 50 seeds. The gerboxes were incubated in biochemical oxygen demand (BOD) at a temperature of 20 ± 2 °C for 24 hours, with a photoperiod of 12 h light/12 h dark. Subsequently, the gerboxes were transferred to the freezer at -20 ± 2 °C for 24 hours, and then incubated again at 20 ± 2 °C for seven days. The fungi present in the seeds were identified using a stereoscopic microscope and a common optical microscope to quantify the incidence of fungi in percentage.

Mycelial growth inhibition of *Alternaria alternata in vitro* with nanoparticles and photodynamic therapy

The efficacy of NPs and PhT with MB and TB dyes in inhibiting the mycelial growth of *A. alternata* was determined using spores from naturally infested seeds cultivated in Petri dishes containing potato dextrose agar (PDA) medium at 28 ºC for seven days. Subsequently, 5 mm diameter discs were detached from the medium containing the mycelium.

In Petri dishes containing the PDA medium were added 1 mL of ZnO, ZnO:1Ag, ZnO:1Cu, ZnOCl, ZnOCl:0.1Ag, and ZnOCl:1Cu NP solutions at concentrations of 2.5 and 5 mg mL^{-1} in sterile distilled water, Carboxin + Thiram (Vitavax) (1 mL 6 mL^{-1} water), and sterile distilled water (control) homogenised using a Drigalski spatula. Then, the mycelium discs containing the fungus were placed on the medium.

PhT was performed using MB, TB, and MB + TB at concentrations of 50 and 100 µmol L^{-1} in saline solution (NaCl 0.45%) sterilised in an autoclave for 20 minutes at 120 ºC. In Petri dishes containing PDA were added 1 ml of the dye solutions, Carboxin + Thiram, and saline solution (NaCl 0.45%, control). Then, the mycelium discs containing the fungus were placed on the medium. The dishes were covered with aluminium foil and kept for 20 minutes at room temperature (25 °C). Then, the dish lids were opened in laminar flow, and irradiated with LED

light (650 nm) for 20 minutes (Ferreira; Tebaldi; Oliveira, 2021), then the plates were incubated in BOD at 28 °C for seven days.

The percentage *A. alternata* mycelial growth inhibition was evaluated using the Equation 1.

$$PGI = \frac{C - T}{C} \times 100 \quad (1)$$

where PGI = percentage of mycelial growth inhibition, C = control diameter (mm), T = treatment diameter (mm) (Nascimento et al., 2013).

The experimental design was completely randomised with four replications, in a 6 x 2 + 2 factorial arrangement (6 NPs, 2 concentrations + 2 additional) for NPs and a 3 x 2 + 2 factorial arrangement (3 dyes x 2 concentrations + 2 additional) for PhT. The data were subjected to normality (Lilliefors-corrected Kolmogorov-Smirnov) and homogeneity tests (Levene). The means were compared by the Scott-Knott test at 5% probability using the RStudio (2015) software.

Treatment of wheat seeds with nanoparticles and photodynamic therapy

Wheat seeds naturally contaminated with *A. alternata* were treated with ZnO, ZnO:1Ag, ZnO:1Cu, ZnOCl, ZnOCl:0.1Ag, and ZnOCl:1Cu NPs at concentrations 2.5 and 5 mg mL^{-1} in sterile distilled water, Carboxin + Thiram (3 mL Kg of $seeds^{-1}$), and sterile distilled water (control). The wheat seeds (1,200) and 120 mL of each treatment were placed in 400 mL beakers, remaining in the solution for ten minutes. Then the seeds were dried on filter paper in laminar flow.

For the PhT, 1,200 wheat seeds of each treatment were placed in 400 mL beakers and added with 120 mL of MB, TB, and MB + TB dyes at concentrations of 50 and 100 µmol L^{-1} in saline solution (NaCl 0.45%) (Ferreira; Tebaldi; Oliveira, 2021), Carboxin + Thiram, and saline solution (NaCl 0.45%, control). The beakers were covered with aluminium foil and kept for 20 minutes at room temperature (25 °C), then the aluminium foil was removed and the seeds were irradiated with LED light for 20 minutes.

The sanitary quality of wheat seeds treated with NPs and with PhT was evaluated according to the method described above. The percentage of fungus control in the seeds was determined by the Equation 2.

$$C = \frac{I - T}{I} \times 100 \quad (2)$$

where C = control percentage, I = incidence of the fungus in the control (%), T = incidence of the fungus in the treatment (%).

The standard wheat seed germination test was performed according to the Seed Analysis Rules (Brasil, 2009) using 200 seeds for each treatment, with four replications of 50 seeds. The seeds were placed on two sheets of Germitest paper moistened with distilled water at a rate of 2.5-fold the weight of the dry paper and placed to germinate at 20 ± 1 ºC for eight days with a photoperiod of 12h light/12h dark. The results were expressed in percentage (%) of germination. The strong normal seedling (SNS) test evaluated the vigour of the seeds eight days after the beginning of the standard germination test. Seedlings with normal aspect were registered and the result expressed in % SNS (Nakagawa, 1999). From the standard germination test, the seedlings that presented 1 mm of radicle emission were evaluated daily until the eighth day of the test to determine the germination speed index (GSI) of the seeds using the Equation 3.

$$GSI = \frac{N1}{D1} + \frac{N2}{D2} + \ldots \frac{Nn}{Nn} \quad (3)$$

where GSI = germination speed index, N = number of seedlings that presented 1 mm of radicle emission, D = number of days after installation of the test (Maguire, 1962).

A total of 200 wheat seeds were used for seedling emergence in four replications of 50 seeds. The seeds were sown in trays containing sand at their field capacity, in a greenhouse. Seedling emergence was evaluated by the number of seeds emerged over ten days by calculating the percentage of seedling emergence (Brasil, 2009). The seedling emergence speed index (ESI) was calculated from the number of seedlings emerged from the first to the tenth day of sowing using the Equation 4.

$$ESI = \frac{N1}{D1} + \frac{N2}{D2} + \ldots \frac{Nn}{Nn} \quad (4)$$

where, N = number of emerged plants, D = number of days after sowing (Maguire, 1962).

The accelerated ageing test included 600 wheat seeds of each treatment uniformly distributed on a stainless-steel mesh inside each Gerbox-type box. Previously, each box was added with 40 mL of distilled water and taken to the ageing chamber at 43 ± 1 °C for 48 hours (Ohlson et al., 2010). Then, the standard germination test was performed, as described above.

The experimental design used for sanitary quality and for all tests of physiological seed quality was in randomised blocks with four replications, in a 6 x 2 + 2 factorial arrangement (6 NPs x 2 concentrations + 2 additional) for NPs and 3 x 2 + 2 (3 dyes x 2 concentrations + 2 additional) for PhT, and the data were subjected to normality (Lilliefors-corrected Kolmogorov-Smirnov) and homogeneity tests (Levene). The means were compared by the Scott-Knott test at 5% probability using the RStudio (2015) software.

RESULTS AND DISCUSSION

The evaluation of the initial sanitary quality of the wheat seeds showed incidence of the fungi *Alternaria alternata* (97%), *Bipolaris sorokiniana* (12%), *Epicoccum* sp. (10%), *Cladosporium cladosporioides* (3%), *Colletotrichum graminicola* (0.7%), and *Fusarium graminearum* (0.6%). Wheat seeds are host to more than 20 genera of fungi (Kobayasti; Pires, 2011), therefore, it is essential to sow pathogen-free seeds.

The ZnO, ZnO:1Ag, ZnO:1Cu, ZnOCl, ZnOCl:0.1Ag, and ZnOCl:1Cu NPs inhibited the mycelial growth of *A. alternata* at concentrations of 2.5 and 5 mg mL^{-1} (Table 1) with results ranging from 24 to 54% and 39 to 79%, respectively, differing significantly from the control (0%). The ZnO:1Ag NP at 5 mg mL^{-1} showed a 79% inhibition of mycelial growth, differing from the other NPs. The fungicide Carboxin + Thiram showed a mycelial growth inhibition of 95%, significantly differing from the other treatments. The inhibition of *A. alternata* growth differed significantly with the use of NPs at a concentration of 5 mg mL^{-1} (56%) and 2.5 mg mL^{-1} (36%).

The fungicidal effect of NPs has been described, such as the use of barium ferrite (BaFe) NP in inhibiting the mycelial growth of *A. alternata, Fusarium oxysporum, Colletotrichum gloeosporioides,* and *Marssonina rosae* (Thakur et al., 2020) and of silver NP for *Aspergillus flavus* (Villamizar-Gallardo; Cruz; Ortíz-Rodriguez, 2016).

In PhT, MB, TB and MB + TB dyes inhibited the mycelial growth of *A. alternata* at concentrations of 50 and 100 µmol L^{-1} (Table 2) with results ranging from 57 to 71% and from 59 to 87%, respectively, differing significantly from the control (0%). MB + TM at 100 µmol L^{-1} (87 %) and Carboxin + Thiram (95%) showed no significant difference in inhibiting mycelial growth; however, they differed from the other treatments. The use of the dyes at the concentration of 100 µmol L^{-1} (72%) differed significantly from the concentration of 50 µmol L^{-1} (64%) in inhibiting mycelial growth.

Table 1: Mycelial growth inhibition percentage of *Alternaria alternata* with different nanoparticles at two concentrations (mg mL^{-1}).

Nanoparticles	Mycelial growth inhibition (%)	
	2.5	5
ZnO	31 dA	39 dA
ZnO:1Ag	54 bB	79 bA
ZnO:1Cu	40 cB	57 cA
ZnOCl	35 dB	69 cA
ZnOCl:0.1Ag	24 dB	48 dA
ZnOCl:1Cu	29 dA	42 dA
Carboxin + Thiram	95 a	
Control	0 e	
Mean (Nanoparticles)	36 B	56 A
CV (%)	15.38	

Means followed by different lowercase letters in the column and uppercase letters in the row differ among themselves by the Scott-Knott test ($p < 0.05$).

Table 2: Photodynamic therapy in the mycelial growth inhibition percentage of *Alternaria alternata in vitro* with methylene blue and toluidine blue dyes at two concentrations (µmol L^{-1}).

Dyes	Mycelial growth inhibition (%)	
	50	100
MB	57 cB	70 bA
TB	65 bA	59 cA
MB + TB	71 bB	87 aA
Carboxin + Thiram	95 a	
Control (NaCl)	0 d	
Mean (Dyes)	64 B	72 A
CV (%)	9.1	

Means followed by different lowercase letters in the column and uppercase letters in the row differ among themselves by the Scott-Knott test ($p < 0.05$).

The fungicidal action of the MB dye was described in controlling the fungus *Trichophyton mentagrophytes*, as the reactive oxygen species generated by PhT kills the pathogen (López-Chicón et al., 2016). The MB, TB, and MB + TB dyes also inhibited the *in vitro* growth of *X. gardneri* (Ferreira; Tebaldi; Oliveira, 2021).

Figure 1 shows the mycelial growth of *A. alternata* in PDA culture medium (A), and the inhibition of mycelial growth with the use of the fungicide Carboxin + Thiram (B), ZnO: 1Ag NP (C), and MB + TB dyes at 100 µmol L^{-1} (D).

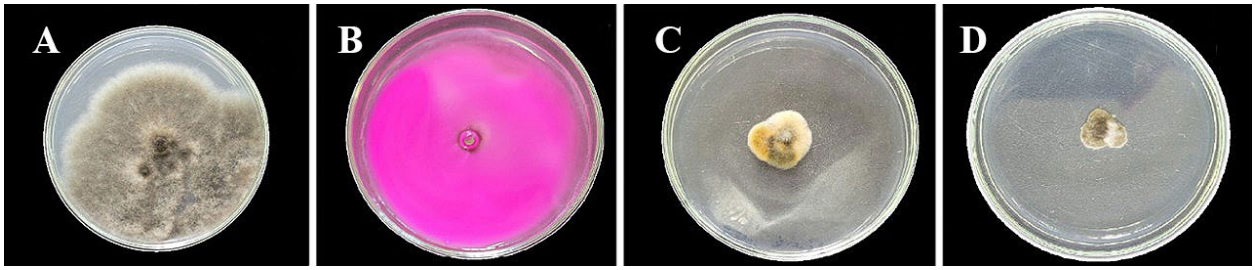

Figure 1: Mycelial growth of *Alternaria alternata* in PDA culture medium (A). Mycelial growth inhibition in culture medium containing Carboxin + Thiram (B), ZnO:1Ag nanoparticle (C), and methylene blue + toluidine blue at 100 µmol L^{-1} (D).

Wheat seeds treated with the ZnO, ZnO:1Ag, ZnO:1Cu, ZnOCl, ZnOCl:0.1Ag, and ZnOCl:1Cu NPs (Table 3) showed no significant difference between the concentrations 2.5 (58%) and 5 mg mL^{-1} (59%) in the incidence of *A. alternata*. Seeds treated with the different NPs showed a fungus incidence of 66 to 51%, corresponding to 28 to 45% pathogen control, respectively, significantly differing from the control (92%). Seeds treated with Carboxin + Thiram showed a 1% incidence of fungus, corresponding to 99% control.

Table 3: *Alternaria alternata* incidence and control in naturally contaminated wheat seeds treated with different nanoparticles at two concentrations (mg mL^{-1}).

Nanoparticles	Incidence (%)		Control (%)	
	2.5	5	2.5	5
ZnO	55 bA	61 bA	40 bA	34 bA
ZnO:1Ag	51 bA	65 bA	45 bA	29 bA
ZnO:1Cu	66 bA	54 bA	28 bA	41 bA
ZnOCl	55 bA	60 bA	40 bA	35 bA
ZnOCl:0.1Ag	66 bA	57 bA	28 bA	38 bA
ZnOCl:1Cu	55 bA	58 bA	40 bA	37 bA
Carboxin + Thiram	1 a		99 a	
Control	92 c		0 c	
Mean (Incidence and Control)	58 A	59 A	37 A	36 A
CV (%)	18.87			

Means followed by different lowercase letters in the column and uppercase letters in the row differ among themselves by the Scott-Knott test ($p < 0.05$).

The inhibition of *A. alternata* mycelial growth by NPs showed a significant difference between the concentrations of 2.5 and 5 mg mL^{-1}; however, there was no difference in seed treatment, with both concentrations showing similar control. A hypothesis for the different controls between NPs and the fungicide in the treatment of wheat seeds is the low uniformity of NP adherence to the seed surface, in addition to the severity of the pathogen in the seeds, which presented 100% fungus colonisation. The use of silver NPs in the treatment of soybean seeds showed 90% control of *Phomopsis* sp. due to the destruction of the cell membrane of the fungus (Mendes et al., 2014).

In PhT (Table 4), wheat seeds treated with MB, TB, and MB + TB dyes showed a significantly different incidence of *A. alternata* between the concentrations of 50 µmol L^{-1} (47%) and 100 µmol L^{-1} (25%). At a concentration of 50 µmol L^{-1}, there was a significant difference between the dyes (34 to 64%), the fungicide (1%), and the control (89%) in the incidence of the fungus in the seeds, with a control of 28 to 62% for the dyes and 99% for the fungicide. At a concentration of 100 µmol L^{-1}, there was no significant difference between the dyes, with the incidence of the fungus varying from 22 to 28%, corresponding to a control of 75 to 69%. The dyes used at a concentration of 100 µmol L^{-1} were effective in reducing the incidence of *A. alternata* in wheat seeds, showing potential use for seed treatment.

The use of MB and TB dyes reduced the incidence of *Candida albicans* in the oral cavity (Rossoni et al., 2010; Campos et al., 2021) and controlled *Fusarium* spp. The efficiency of the dyes can be due to the damage caused by PhT to the fungal cell membrane (Paziani et al., 2019).

Different ZnO, ZnO:1Ag, ZnO:1Cu, ZnOCl, ZnOCl:0.1Ag, and ZnOCl:1Cu NPs at concentrations of 2.5 and 5 mg mL^{-1} (Table 5) did not influence the physiological quality of wheat seeds, with germination ranging from 97 to 99%, strong normal plants ranging

from 97 to 98%, GSI of 24, emergence ranging from 92 to 97%, and ESI of 15 to 16. However, in accelerated ageing, the ZnO, ZnO:1Ag, and ZnO:1Cu NPs significantly reduced the germination of wheat seeds. The fungicide Carboxin + Thiram reduced the physiological quality of the seeds, with the exception of emergence. The use of NPs at concentrations of 2.5 and 5 mg mL^{-1}showed no significant difference in the physiological quality of the seeds. The use of ZnO:0.5Mg, ZnO:1K, and ZnO:1Mg NPs at a concentration of 5 mg mL^{-1} also did not reduce the germination of tomato seeds in controlling *Xanthomonas gardneri* (Fraga et al., 2021). The NPs demonstrated that they can be used in the treatment of wheat seeds, as they did not present a fitotoxic effect on the seeds.

In PhT (Table 6), the dyes MB, TB, and MB + TB at concentrations of 50 and 100 µmol L^{-1} showed no influence on the physiological quality of wheat seeds, with germination of 99%, strong normal plants ranging from 97 to 98%, GSI of 24, emergence ranging from 94 to 96%, and ESI of 15 to 16, not differing from the controls. However, in accelerated ageing, the dyes significantly reduced the germination of wheat seeds. Only Carboxin + Thiram significantly reduced the physiological quality of the seeds. The use of MB and TB dyes had no toxic effect on wheat seeds. According to Ferreira, Tebaldi and Oliveira (2021), MB, TB, and MB + TB also showed no fitotoxicity on tomato seeds.

Wheat seeds treated with Carboxin + Thiram reduced seed germination. Similar results were also observed in reducing germination in oat and rye seeds treated with Thiram (Balardin; Loch, 1987) and in wheat seeds treated with Carboxin (Barros; Salgado; Lasca, 1983).

Therefore, NPs and PhT did not affect the physiological quality of seeds and controlled the fungus *A. alternata* in wheat seeds, demonstrating potential use in the treatment and control of the pathogen in wheat seeds.

Table 4: Photodynamic therapy on the physiological quality of wheat seeds treated with methylene blue and toluidine blue dyes at two concentrations (µmol L^{-1}).

Treatments	Incidence (%)		Control (%)	
	50	100	50	100
MB	34 bA	26 bA	62 bA	71 bA
TB	64 dB	28 bA	28 dB	69 bA
MB + TB	44 cB	22 bA	51 cB	75 bA
Carboxin + Thiram	1 a		99 a	
Control (NaCl)	89 d		0 d	
Mean (Dyes)	47 B	25 A	47 B	72 A
CV (%)	21.73			

Means followed by different lowercase letters in the column and uppercase letters in the row differ among themselves by the Scott-Knott test ($p < 0.05$).

Table 5: Physiological quality of wheat seeds treated with different nanoparticles at two concentrations (mg mL^{-1}).

Nanoparticles	G (%)		SNS (%)		GSI		E (%)		ESI		AA (%)	
	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5
ZnO	99 aA	99 aA	97 aA	98 aA	24 aA	24 aA	97 aA	95 aA	15 aA	15 aA	71 bA	69 bA
ZnO:1Ag	99 aA	98 aA	98 aA	97 aA	24 aA	24 aA	94 aA	92 aA	15 aA	15 aA	65 bA	68 bA
ZnO:1Cu	97 aA	99 aA	97 aA	98 aA	24 aA	24 aA	94 aA	96 aA	16 aA	15 aA	60 cA	59 cA
ZnOCl	99 aA	99 aA	97 aA	97 aA	24 aA	24 aA	98 aA	96 aA	15 aA	15 aA	98 aA	97 aA
ZnOCl:0.1Ag	99 aA	99 aA	97 aA	97 aA	24 aA	24 aA	95 aA	96 aA	15 aA	16 aA	98 aA	98 aA
ZnOCl:1Cu	99 aA	99 aA	98 aA	97 aA	24 aA	24 aA	96 aA	96 aA	15 aA	15 aA	98 aA	97 aA
Carboxin + Thiram	85 b		81 b		17 b		93a.		12 b		23 d	
Control	99 a		97 a		24 a		99 a		16 a		96 a	
CV (%)	3.71		1.21		0.95		3.68		4.66		5.07	

G = Germination; SNS = Normal Strong Seedlings; GSI = Germination Speed Index; E = Emergence; ESI = Emergence Speed Index; AA = Accelerated Ageing. Means followed by different lowercase letters in the column and uppercase letters in the row differ among themselves by the Scott-Knott test ($p < 0.05$).

Table 6: Photodynamic therapy on the physiological quality of wheat seeds treated with methylene blue and toluidine blue dyes at two concentrations (µmol L^{-1}).

Treatments	G (%)		SNS (%)		GSI		E (%)		ESI		AA (%)	
	50	100	50	100	50	100	50	100	50	100	50	100
MB	99 aA	99 aA	98 aA	97 aA	24 aA	24 aA	96 aA	96 aA	16 aA	15 aA	71 bA	80 bA
TB	99 aA	99 aA	97 aA	97 aA	24 aA	24 aA	95 aA	96 aA	15 aA	16 aA	69 bA	73 bA
MB + TB	99 aA	99 aA	98 aA	98 aA	24 aA	24 aA	94 aA	95 aA	15 aA	15 aA	72 bA	81 bA
Carboxin + Thiram	85 b		81 b		17 b		92 b		12 b		23 c	
Control (NaCl)	99 a		97 a		24 a		97 a		16 a		96 a	
CV (%)	0.97		1.21		0.87		3.6		4.35		10.35	

G = Germination; SNS = Normal Strong Seedlings; GSI = Germination Speed Index; E = Emergence; ESI = Emergence Speed Index; AA = Accelerated Ageing. Means followed by different lowercase letters in the column and uppercase letters in the row differ among themselves by the Scott-Knott test ($p < 0.05$).

CONCLUSIONS

The ZnO, ZnO:1Ag, ZnO:1Cu, ZnOCl, ZnOCl:0.1Ag, and ZnOCl:1Cu NPs at concentrations of 2.5 and 5 mg mL^{-1}, and the MB, TB, and MB + TB dyes at concentrations of 50 and 100 µmol L^{-1} inhibited the mycelial growth of *A. alternata,* reduced the incidence of the pathogen in wheat seeds, and did not affect the seeds physiological quality. The ZnO:1Ag NP at 5 mg mL^{-1} and the MB + TB dye at 100 µmol L^{-1} were the most effective in inhibiting mycelial growth.

AUTHOR CONTRIBUTION

Conceptual idea: Tebaldi, N.D.; Catão H.C.R.M.; Methodology design: Tebaldi, N.D.; Catão H.C.R.M.; Duarte, L.C.; Data collection: Duarte, L.C.; Data analysis and interpretation: Tebaldi, N.D.; Catão H.C.R.M.; Duarte, L.C., and Writing and editing: Duarte, L.C.; Tebaldi, N.D.

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