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Toxicity of some nanomaterials against Botrytis allii, the causal pathogen of neck rot disease of onion (*Allium cepa* L.)

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ABSTRACT

In this study, 14 fungal isolates associated with symptomatic onion samples collected from different counties of Sohag Governorate showing neck rot disease were isolated and identified as *Botrytis allii* Munn (12 isolates) and 2 isolates of B. *cinerea* Pers. Pathogenicity tests carried out in the greenhouse showed that only the isolates of B. *allii* were pathogenic to onion plants and caused identical neck rot symptoms among all tested fungal isolates. However, B. *allii* isolates exhibited different levels of virulence. Isolate No. 9 of B. *allii* was the highest pathogenic one. *In vitro*, the influence of carbon, MgO, and ZnO nanoparticles was tested at 0.1, 0.2, and 0.3 mM on mycelial linear growth and biomass of B. *allii*. Results obtained showed that all tested nanomaterials significantly reduced the mycelial linear growth and biomass at all tested concentrations. The nano MgO at 0.3 mM caused the high inhibitory effect followed by nano ZnO, where they highly reduced mycelial linear growth and biomass compared to the untreated controls. In greenhouse and field experiments, the MgO and ZnO nanoparticles at 0.3 mM highly reduced the disease incidence and severity compared with the control of untreated plants. Moreover, the MgO and ZnO nanoparticles at 0.3 mM highly reduced the neck rot incidence and the decline in bulb weight of onion due to infection with B. *allii* during storage. Therefore, this study reports that the MgO and ZnO nanoparticles are eco-friendly alternative approaches for suppressing neck rot disease on onion in the field and storage.

Keywords:
Onion, neck rot, *Botrytis allii*, toxicity, nanomaterials, control.

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INTRODUCTION

Onion (*Allium cepa* L.) is considered one of the most important economic vegetable crops worldwide. Onion cultivation increased considerably during the last ten years in Egypt, where the total acreage of onion in 2019 reached 209,400 feddan with a total yield of 3,081,047 tons and an average yield of 16.219 tons/feddan (FAOSTAT, 2021). Unfortunately, onion is subjected to be attacked with various pathogens during all stages of onion production, which affects its health and yield (Fritsch and Friesen, 2002). In Egypt, onions are most infected with fungal diseases than any other diseases due to climatic conditions suitable to infect the plant (Abdalla *et al.*, 2019). Among these fungal diseases, neck rot disease, caused by a complex of Botrytis spp., is an important fungal disease of onion worldwide, especially after harvesting, causing severe bulb losses during storage (Lacy and Lorbeer 2008). The disease is mainly caused by one or more of the following species of the genus Botrytis included B. aclada Fresen (syn. B. *allii* Munn), B. squamosa J.C. Walker, and B. cinerea Pers. (Köhl *et al.*, 1991; Brewster, 1994; Jorjandi *et al.*, 2009). Most of the bulb losses have occurred in temperate regions, where B. *allii* was the principal species affecting onions as the common causal pathogen appeared of rotted onion, as reported by Nielsen *et al.* (2001) and du Toit *et al.* (2002). In the field, infected plants exhibit leaf distortion, stunted growth, and splitting of leaves around the neck area. However, infection with B. *allii* usually remains symptomless in leaves, and the fungus grows from the leaves into the bulb during curing, leading to neck rots of bulbs in storage (Lacy and Lorbeer, 2008). In addition, the pathogen conidiophores and sclerotia, and neck rots on bulbs are occasionally noted in the field (Chilvers *et al.*, 2004b). Conidia of the fungus arise from these sclerotia of infected plants and are carried out by wind to spread the disease (Muimba-Kankolongo, 2018). Traditional methods for controlling neck rot of onion using fungicide applications are harmful to humans and the environment. Therefore, it has become necessary to search for safe, eco-friendly alternative approaches for control plant diseases.

In recent years, nanoparticle (NP) materials have received increasing attention due to their unique physical and chemical properties, which differ significantly from their conventional counterparts (Stoimenov *et al.*, 2002). Thus, the NP materials could be eco-friendly alternative approaches instead of chemicals for suppressing plant diseases. Recent studies have demonstrated the antifungal activity of various NP materials, including carbon (Wang *et al.*, 2014), magnesium oxide (Parizi *et al.*, 2014; El-Argawy *et al.*, 2017; De la Rosa-García *et al.*, 2018; Chen *et al.*, 2020), and zinc oxide (He *et al.*, 2011; Wani *et al.*, 2012; De la Rosa-García *et al.*, 2018). The single-walled carbon nanotubes at 500 µg mL^{-1} showed antifungal activity and highly reduced the mycelial dry weight of Fusarium graminearum and F. poae (Wang *et al.*, 2014). The ZnO NP at concentrations greater than 3 mmole L^{-1} significantly inhibited the growth of B. cinerea and Penicillium expansum (He *et al.*, 2011), and MgO and ZnO nanoparticles at 100 ppm highly reduced the mycelial radial growth of F. oxysporum f. sp. lycopersici and F. oxysporum f.sp. betae (Parizi *et al.*, 2014; El-Argawy *et al.*, 2017). Also, ZnO and MgO nanoparticles caused a significant inhibition of the germination of the spores of Alternaria alternata, F. oxysporum, Rhizopus stolonifer, and Mucor plumbeus (Wani *et al.*, 2012) and Colletotrichum gloeosporioides (De la Rosa-García *et al.*, 2018). In a recent study, nano MgO inhibited the fungal growth of Thielaviopsis basicola and spore germination of Phytophthora nicotianae (Chen *et al.*, 2020). Moreover, nanoparticles also were found to play a unique role in the direct uptake and high accumulation of silica, leading towards the erecting of the leaves and improving the defense of fungal pathogens (Suriyaprabha *et al.*, 2012). In previous studies, these NP materials have been effectively controlled various plant fungal pathogens on several economical plants (He *et al.*, 2011; Parizi *et al.*, 2014; Wang et al., 2014; El-Argawy *et al.*, 2017; De la Rosa-García *et al.*, 2018; Chen *et al.*, 2020). As available information, no reports documented the effect of carbon, magnesium oxide, and zinc oxide nanoparticles on the growth of B. *allii* and the development of neck rot disease of onion in the field and storage. Therefore, the objective of the current study was to evaluate in vitro the antifungal activity of carbon, magnesium oxide, and zinc oxide nanoparticles tested at different concentrations against B. *allii* causing onion neck rot disease. Also, the control efficacy on the neck rot incidence in the greenhouse and under field conditions and bulb weight during storage were investigated.

MATERIALS AND METHODS

1. Isolation and identification of the causal pathogen of onion neck rot disease

Samples of infected onion plants showing neck rot symptoms were collected from Juhanah, Gerga, Alasairat, Dar El Salam, El Balyana, El Munshah, Sohag, Akhmem, Saqultah, El Maragha, Tahta, and Tema of Sohag Governorate, Egypt. Infected neck parts were cut into small pieces and washed thoroughly with tap water. Pieces were surface sterilized with 1% sodium hypochlorite (SH) solution for 3 min and then washed three times thoroughly in sterilized distilled water (SDW). Then pieces were dried between two folds of sterilized filter papers (SFP). Every three pieces were placed into 9.0 cm Petri dishes containing Potato Dextrose Agar (PDA) medium supplemented with streptomycin sulfate (400 mg L-1 of medium). Then Petri dishes were incubated at 20 ºC and examined daily to observe the hyphal growth. The growing fungal colonies were purified by single spore and hyphal tip techniques, followed by sub-culturing onto a freshly prepared PDA medium at the same conditions. The pure fungal isolates obtained were identified according to the morphological characteristics of mycelia, conidiophores, conidiospores, and sclerotia described by Kritzman (1983), Chilvers *et al*. (2004a), Chilvers and du Toit (2006) then confirmed in the Assiut University Mycology Center. Stock cultures of all fungal isolates were maintained at 5 °C on PDA slants for further studies.

2. Pathogenicity tests

The pathogenic capabilities of 14 fungal isolates were tested on the Giza 20 onion cultivar in the open greenhouse during the 2016/2017 growing season at the Shandaweel Agricultural Research Station, Sohag Governorate. Onion seedlings (45-day-old) were surface sterilized by dipping in 0.1% SH solution for 3 min, washed three times with SDW, and then left for drying at room temperature (Sayed *et al.*, 2014). The inoculum of each fungal isolate was prepared by placing two disks (0.5 cm in diameter) taken from the 7-day-old culture into conical flasks containing 100 ml autoclaved PDA broth medium. Then flasks were placed on a rotary checker at 3.000 rpm and 20±0.5 °C for 2 weeks. The fungal growth of each isolate was collected by filtering the growth on SFP, washed several times with SDW, and then blended in 100 ml SDW for 30 sec to get an even spore suspension using a sterilized blender. Each spore suspension was then adjusted to 104 spores ml-1 using a hemocytometer and supplied with 50 mg of Carbenicillin antibiotic to suppress bacterial contamination (Lin *et al.*, 1995). Formalin-sterilized plastic pots (30 cm in diameter) were filled with formalin-sterilized loam soil (5.0 kg of each pot), planted with 5 onion seedlings per pot, and then irrigated every other day. Later 60 days of transplanting, the neck of onion bulbs were inoculated by spraying 10 ml of each isolate's spore suspension using a hand atomizer (Kaufman and Lorbeer, 1967). An equal amount of SDW was applied to the neck bulbs of the control treatment. The experiment was performed with three pots (replicates) of each isolate tested in a completely randomized design. One month after inoculation, visual observations of neck rot symptoms were recorded, and the individual bulbs were rated for the disease severity (DS) using a scale of 0-4: where 0 = no rot, 1= rot only close to the neck, 2 = upper third with rot, 3 = upper two thirds with rot, 4 = more than two-thirds with rot (Köhl *et al.*, 1991). The percentages of disease incidence (DI) and disease severity were calculated using the following formulae:

$$DI\% = \text{No. of infected plants} / (\text{Total plants} \times 100)$$

$$DS\% = (\Sigma\ Si \times Ni) / (4 \times Nt) \times 100$$

Si is the severity ratings 0-4, Ni is the number of plants in each ratting, and Nt is the total number of rated plants.

Also, the main causal pathogen of neck rot was consistently re-isolated from infected tissue of onion plants.

3. Effect of some nanomaterials s on the mycelial growth of B. *allii in vitro*

Nanomaterials of carbon, magnesium oxide (MgO), and zinc oxide (ZnO) were obtained from the Department of Plant Physiology, Faculty of Agriculture Cairo University. According to the source, nanomaterials' particle sizes ranged from 18 to 49 nm and were spherical in shape after the

UV spectral analysis and transmission electron microscopy examination. Each nanomaterial (1 mM stock solution) was incorporated into the PDA medium before solidification in the conical flasks to obtain the final concentrations of 0.1, 0.2, and 0.3 mM, and the medium was then poured into 9.0 cm Petri dishes. Then dishes were inoculated in the center with 5-mm discs of B. *allii* obtained from the 7-day-old culture and incubated at 20 °C till the control dishes were wholly covered with mycelium. Inoculated dishes without nanomaterials served as a control, and four dishes (replicates) were used for each treatment. The diameter of mycelium linear growth (cm) was measured. Otherwise, the incorporated potato dextrose (PD) broth medium with the same concentrations in the conical flasks and inoculated with 5-mm B. *allii* discs were placed on a rotary shaker at 20 °C. After ten days of shake culture, the biomass was separated from the culture broth by filtration, dried for 24 h at 70 °C, weighed (mg), and the means were calculated.

4. Effect of some nanomaterials on the infection with B. *allii* under greenhouse conditions

The following experiments were carried out in the greenhouse of Shandaweel Island Agricultural Research Station, Sohag governorate, during the 2017/2018 and 2018/2019 growing seasons. Onion seedlings (45-day-old) were surface sterilized by dipping in 0.1% SH solution for 3 min, washed three times with SDW, and then left for drying at room temperature. The inoculum of B. *allii* isolate No. 9 was prepared as mentioned before. The seedlings of onion cv Giza 20 were treated with nano carbon, nano MgO, and nano ZnO at different concentrations of 0.0, 0.1, 0.2, and 0.3 mM of each by spraying 10 ml of each concentration per seedling ten days before inoculation with B. *allii*. Six pots per concentration of each nanomaterial tested were used as replicates in a completely randomized block design, and 5 seedlings were in each pot. Later 60 days of transplanting, the neck of onion bulbs were inoculated with B. *allii* inoculum using a hand atomizer. One month after inoculation, the percentages of DI and DS have been recorded, as mentioned before. The means over the two growing seasons were then calculated and statically analyzed, as mentioned before.

5. Effect of some nanomaterials on the infection with B. *allii* under field conditions

Under filed conditions and artificial infestation, the following experiments were conducted at the Shandaweel Island Agriculture Research Station, Sohag, Governorate, during the 2017/2018 and 2018/2019 growing seasons. Seeds of onion cv Giza 20 were planted in the nursery on the 25th of September for 60 days which received all recommended care conditions for producing onion seedlings. A Complete Randomized Split-plot Design has followed some experiments where the main plots assembled the number of nanomaterials applied before inoculation with B. *allii* as mentioned before, and the subplots were used for the concentrations of each nanomaterial. Three plots 3 × 3.5 m of each were used as replicates of each treatment, and nontreated plots served as control. Each plot had four rows with 60 cm apart space between rows, and 60 seedlings were planted in each row. All cultural practices recommended for onion production were carefully followed. The inoculum of isolate No. 9 of B. *allii* was prepared as mentioned before. The seedlings of onion cv Giza 20 were treated with nano carbon, nano MgO, and nano ZnO at different concentrations of 0.0, 0.1, 0.2, and 0.3 mM of each by spraying 10 ml of each concentration per seedling ten days before inoculation with B. *allii*, as mentioned before. Later 60 days of transplanting, the neck of onion bulbs in whole plots were inoculated with B. *allii*, as mentioned before. Also, the percentages of DI and DS have been recorded. The means over the two growing seasons were then calculated and statically analyzed, as mentioned before.

6. Effect of nanomaterials on neck rot incidence and bulb weight of onion during storage

After harvesting the field experiments previously conducted for all nanomaterials applied in each season, treated and nontreated yield onion bulbs were collected (separate replicates) and stored without topping at room temperature. The bulbs were then examined after 2 and 4 months of storage to estimate the incidence of neck rot disease. The means of the incidence for all treatments of control agents during storage were then calculated. Also, the bulb's weight readings (kg) were recorded, and the reduction in the bulb weights over the control plants for all treatments of nanomaterials was calculated. The means over the two growing seasons were then calculated and statically analyzed.

Statistical analysis

Data obtained were statistically analyzed by the MSTAT-C program version 2.10. Means were compared by Duncan's multiple range tests, and the least significant difference (LSD) was used at the p = 0.05 level of probability described by Gomez and Gomez (1984).

RESULTS

1. Isolation and identification of the causal pathogen of onion neck rot disease

Isolation from diseased onion plants showing neck rot symptoms collected from Juhanah, Gerga, Alasairat, Dar El Salam, El Balyana, El Munshah, Sohag, Akhmem, Saqultah, El Maragha, Tahta, and Tema of Sohag Governorate resulted in a total of 14 fungal isolates (Table 1). The 14 fungal isolates obtained were identified as Botrytis *allii* Munn (12 isolates) and B. cinerea Pers. (2 isolates).

2. Pathogenicity tests

The pathogenic capability of 12 isolates of B. *allii* and 2 isolates of B. cinerea were tested on the onion Giza 20 cultivar in the open greenhouse during the 2016/2017 growing season. Results presented in Table 2 show that only isolates of B. *allii* were pathogenic to onion bulbs and caused identical neck rot symptoms among all isolates tested. Isolate No. 9 of B. *allii* was the high pathogenic one and caused disease incidence and severity of 95 and 81.65%, respectively. While isolate No. 4 was the lowest pathogenic one and caused DI and DS of 60 and 34.15%. The other isolates of B. *allii* caused DI ranging from 65 to 75% and DS from 44.14 to 53.75%. On the other hand, both isolates of B. cinerea tested induced slightly rotted necks associated with grey color.

3. Effect of some nanomaterials on the mycelial growth of B. *allii in vitro*

The influence of carbon, MgO, and ZnO nanoparticles at different concentrations 0.0, 0.1, 0.2, and 0.3 mM on mycelial linear growth and biomass of B. *allii* was studied. Data presented in Table 3 and Figure 1 show that all nanomaterials significantly decreased the mycelial linear growth and biomass at all tested concentrations. The inhibitory effect increased by increasing the concentration of each nanomaterial tested. The high inhibitory effect on the growth was detected for nano MgO, particularly at 0.3 mM, where it caused a high reduction in mycelial linear growth, and biomass reached 2.37 cm and 0.113 mg compared with 8.85 cm and 0.301 mg in control, respectively. While nano carbon at 0.3 mM caused the lowest inhibitory effect and decreased the mycelial linear growth and biomass to 6.90 cm and 0.206 mg, respectively, compared to the control. On the other hand, nano ZnO at 0.3 Mm caused 4.47 cm and 0.119 mg of mycelial linear growth and biomass, respectively.

4. Effect of some nanomaterials on the infection with B. *allii* under greenhouse conditions

The efficacy of some nanomaterials tested at different concentrations on infection with B. *allii* causing neck rot disease of onion was studied. Data in Table 4 demonstrate that the tested nanomaterials at different concentrations significantly varied in their effectiveness on the incidence of neck rot disease of onion. Moreover, the efficiency increased by increasing the concentration of each nanomaterial tested. The high efficiency of nanomaterials was detected for nano MgO, particularly at 0.3 mM. It caused a high reduction in DI and DS reached 24.66 and 20.66%, respectively, compared with 95.33 and 69.66% of the control. While nano carbon at 0.3 mM caused the lowest efficacy and slightly reduced the DI and DS to 85.33 and 63.33%, respectively, compared to the control. On the other hand, nano ZnO at 0.3 Mm highly reduced DI and DS to 37.33 and 25.33%, respectively. However, the nano MgO was better than nano ZnO in such effect.

5. Effect of some nanomaterials on the infection with B. *allii* under field conditions

Data in Table 5 demonstrate that the nanomaterials tested at different concentrations significantly varied in their effectiveness on the onion incidence of neck rot disease. Moreover, the efficiency increased by increasing the concentration of each nanomaterial tested. The high efficiency of nanomaterials tested was detected for nano MgO, particularly at 0.3 mM. It caused a high reduction in DI and DS reached 24.66 and 20.66%, respectively, compared with

95.33 and 69.66% of the control. While nano carbon at 0.3 mM caused the lowest efficacy and slightly reduced the DI and DS to 85.33 and 63.33%, respectively, compared to the control. On the other hand, nano ZnO at 0.3 Mm highly reduced DI and DS to 37.33 and 25.33%, respectively. However, the nano MgO was better than nano ZnO in such effect.

6. Effect of nanomaterials on neck rot incidence and bulb weight of onion during storage

The efficacy of some nanomaterials tested at different concentrations on the neck rot incidence and reduction in bulb weight during storage after harvesting yield bulbs of the two growing seasons 2017/2018 and 2018/219 was investigated. Data in Table 6 demonstrate that the nanomaterials tested at different concentrations significantly varied in their effectiveness on the onion incidence of neck rot disease and reduction bulb weight during storage. Moreover, the efficiency increased by increasing the concentration of each nanomaterial. The high efficiency of nanomaterials tested was detected for nano MgO, particularly at 0.3 mM, where it caused the lowest DI and reduction in bulb weight during storage reached 12.25% and 10.93%, respectively, compared with 77.75% and 53.81%, respectively of the control. While nano carbon at 0.3 mM caused the lowest efficacy and slightly decreased the DI to 35.75%, and caused a reduction in bulb weight reached 31.74%, compared to the control. On the other hand, nano ZnO at 0.3 Mm highly reduced DI to 37.33% and caused a reduction in bulb weight reached 25.33%. However, the nano MgO was better than nano ZnO in such effect.

Table 1: Source and identification of 14 fungal isolates obtained from diseased onion plants showing neck rot symptoms in Sohag Governorate.

Isolate No.	Source	Identification
1	Juhanah	*Botrytis allii* Munn
2	Gerga	*B. allii* Munn
3	Alasairat	*B. allii* Munn
4	Dar El Salam	*B. allii* Munn
5	Dar El Salam	*B. cinerea* Pers.
6	El Balyana	*B. allii* Munn
7	El Munshah	*B. allii* Munn
8	Sohag	*B. allii* Munn
9	Ahkmem	*B. allii* Munn
10	Saqulta	*B. allii* Munn
11	El Maragha	*B. allii* Munn
12	Tahta	*B. allii* Munn
13	Tema	*B. allii* Munn
14	Tema	*B. cinerea* Pers.

Table 2: Pathogenic capability of 14 isolates of *B. allii* and *B. cinerea* on the onion Giza 20 cv in the open greenhouse during the 2016/2017 growing season.

Isolate No.	*Botrytis* sp.	DI%	DS%
1	*B. allii*	70.00	49.57
2	*B. allii*	75.00	46.65
3	*B. allii*	65.00	45.82
4	*B. allii*	60.00	34.15
5	*B. cinerea*	1.50*	0.20
6	*B. allii*	70.00	56.65
7	*B. allii*	70.00	52.07
8	*B. allii*	75.00	53.75
9	*B. allii*	95.00	81.65
10	*B. allii*	65.00	44.14
11	*B. allii*	75.00	60.82
12	*B. allii*	65.00	52.07
13	*B. allii*	75.00	50.00
14	*B. cinerea*	2.00*	0.50
Control		0.00	0.00
L.S.D. at 5%		18.66	12.39

* Insignificant slightly rotted necks

Table 3 :Effect of some nanomaterials on the mycelial growth of *B. allii*.

Nanomaterials	Concentrations (mM)	Mycelial linear growth (cm)	Biomass (mg)
Nano MgO	0.0	8.85	0.301
	0.1	3.42	0.143
	0.2	2.87	0.125
	0.3	2.37	0.113
Mean		**4.38**	**0.171**
Nano ZnO	0.0	8.85	0.301
	0.1	6.42	0.158
	0.2	4.77	0.145
	0.3	4.47	0.119
Mean		**6.13**	**0.181**
Nano carbon	0.0	8.85	0.301
	0.1	8.10	0.231
	0.2	7.82	0.225
	0.3	6.90	0.206
Mean		**7.92**	**0.241**
General average		**6.14**	**0.198**
$L.S.D._{0.05}$	Nanomaterials (A)	0.347	0.008
	Concentrations (B)	0.272	0.006
	A × B	0.472	0.011

A

B

C

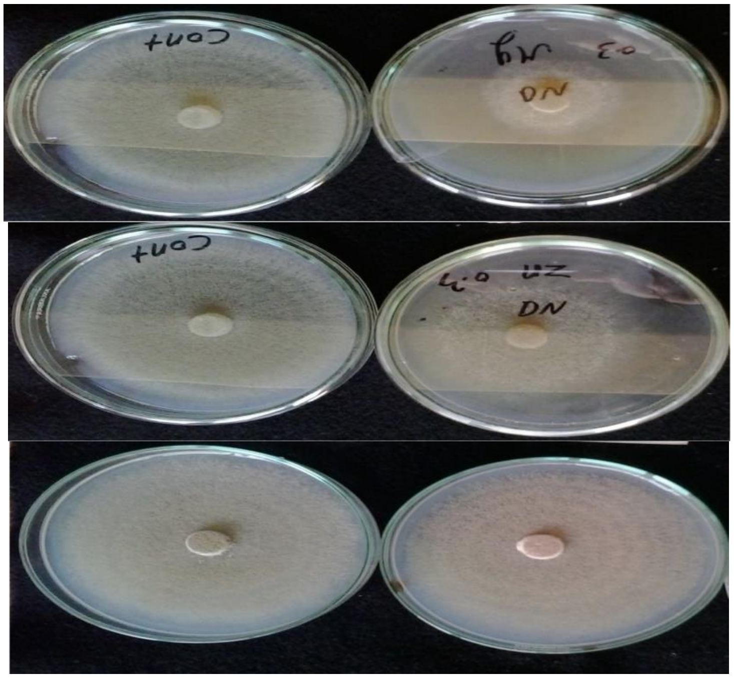

Fig. 1: Effect of some nanomaterials tested at 0.3 mM on mycelial linear growth of *B. allii in vitro*: The left plate is the control, and the right plate is nano MgO (A), nano ZnO (B), and nano carbon (C) treatment.

Table 4 :Effect of some nanomaterials on the infection with *B. allii* in the open greenhouse during the 2017/2018 and 2018/2019 growing seasons.

Nanomaterials	**Concentrations (mM)**	**DI%**	**DS%**
Nano MgO	0.0	95.33*	69.66
	0.1	59.33	39.33
	0.2	33.33	26.66
	0.3	24.66	20.66
Mean		**53.17**	**39.08**
Nano ZnO	0.0	95.33	69.66
	0.1	66.66	44.33
	0.2	59.33	38.66
	0.3	37.33	25.33
Mean		**64.66**	**44.49**
Nano carbon	0.0	95.33	69.66
	0.1	91.33	67.66
	0.2	88.33	65.66
	0.3	85.33	63.33
Mean		**90.08**	**66.58**
General average		**69.30**	**50.05**
L.S.D. $_{0.05}$	Nanomaterials (A)	5.03	5.26
	Concentrations (B)	4.78	4.95
	A × B	1.35	1.78

* The values of the presented data are the means over the two growing seasons.

Table 5 :Effect of some nanomaterials on the infection with *B. allii* under field conditions during the 2017/2018 and 2018/2019 growing seasons.

Nanomaterials	Concentrations (mM)	DI%	DS%
Nano MgO	0.0	92.33*	70.33
	0.1	71.33	55.66
	0.2	66.33	49.33
	0.3	60.33	42.33
Mean		**72.55**	**54.42**
Nano ZnO	0.0	92.33	70.33
	0.1	80.33	60.66
	0.2	73.33	55.33
	0.3	66.66	46.66
Mean		**78.16**	**58.16**
Nano carbon	0.0	92.33	70.33
	0.1	85.33	67.66
	0.2	80.33	65.33
	0.3	76.66	63.33
Mean		**83.66**	**66.66**
General average		**78.12**	**59.75**
L.S.D. $_{0.05}$	Nanomaterials (A)	4.69	3.78
	Concentrations (B)	3.43	2.45
	A × B	1.37	1.78

Table 6 :Effect of some nanomaterials on the neck rot incidence and reduction bulb weight of onion during storage after harvesting yield bulbs of the two growing seasons 2017/2018 and 2018/2019.

Nanomaterials	Concentrations (mM)	DI%	% Reduction in bulb weight
Nano MgO	0.0	77.75*	53.81
	0.1	21.25	18.06
	0.2	17.25	13.62
	0.3	12.25	10.93
Mean		**32.13**	**24.11**
Nano ZnO	0.0	77.75	53.81
	0.1	31.50	29.31
	0.2	25.75	23.31
	0.3	18.75	17.25
Mean		**38.44**	**30.92**
Nano carbon	0.0	77.75	53.81
	0.1	45.75	37.75
	0.2	41.50	33.75
	0.3	35.75	31.75
Mean		**50.19**	**39.27**
General average		**40.25**	**31.43**
L.S.D. $_{0.05}$	Nanomaterials (A)	1.03	1.38
	Concentrations (B)	0.98	1.58
	A × B	1.98	2.39

* The values of the presented data are the means over the two growing seasons.

DISCUSSION

Neck rot disease caused by *B. allii* is an important fungal disease of onion in Egypt and worldwide, especially after harvesting, causing severe bulb losses during storage (Köhl *et al*., 1991; Lacy and Lorbeer 2008; Nielsen *et al.* 2001; Sayed *et al*., 2014; du Toit *et al*. 2002), which survives in the soil or on rotting bulbs as sclerotia (Chilvers *et al.*, 2004b; Muimba-Kankolongo, 2018). In the field, infected plants exhibit leaf distortion, stunted growth, and splitting of leaves around the neck area, and grayish sporulation of the fungus, which may be observed between leaf scales near the neck area (Chilvers *et al.*, 2004b; Muimba-Kankolongo, 2018).

In this study, 14 fungal isolates belonging to two species of the genus *Botrytis* were isolated from diseased onion plants collected from different counties of Sohag Governorate showing neck rot symptoms and identified according to the morphological characteristics described by Kritzman (1983), Chilvers *et al.* (2004a), Chilvers and du Toit (2006) as *Botrytis allii* Munn (12 isolates) and *B. cinerea* Pers. (2 isolates). Koch's postulates were fulfilled by the pathogenicity test carried out in the greenhouse on onion cv Giza 20 and reisolation of the pathogen *B. allii* from symptomatic plants typically showing neck rot similar to that naturally infected. Results showed that all isolates of *B. allii* were pathogenic to onion plants with different levels of virulence. These results are in agreement with those reported by Saleh (2004), Jorjandi *et al.* (2009), Sayed *et al*. (2014), Abdalla *et al*., 2019), who found different levels of virulence between isolates of *B. allii*, which may be due to the genetic structure of each isolate.

An attempt was performed to investigate the efficacy of nanoparticles of carbon, MgO, and ZnO tested at different concentrations on the mycelial linear growth and biomass of *B. allii in vitro.* It was clear from the results obtained that the different concentrations of 0.1, 0.2, 0.3 mM of MgO, ZnO, and carbon nanoparticles caused a significant inhibition in the mycelial linear growth and biomass compared to control. However, the highest inhibition in the mycelial linear growth and biomass was observed at 0.3 mM in MgO nanoparticles, followed by zinc and carbon nanoparticles, respectively. In a previous study, ZnO nanoparticles significantly inhibited the growth of *B. cinerea* at concentrations greater than 3 mM L^{-1} (He *et al.*, 2011). Likewise, in other studies, MgO and ZnO nanoparticles highly reduced the mycelial radial growth of *F. oxysporum* f.sp. *betae* at 100 ppm (El-Argawy *et al.*, 2017), caused a significant inhibition of the germination of the spores of *A. alternata*, *F. oxysporum*, *R. stolonifer*, and *M. plumbeus* (Wani *et al.*, 2012) and *C. gloeosporioides* (De la Rosa-García *et al.*, 2018), and inhibited the fungal growth of *T. basicola* and spore germination of *P. nicotianae* (Chen *et al.*, 2020). Meanwhile, the single-walled carbon nanotubes at 500 µg mL^{-1} reduced the mycelial growth of *F. graminearum* and *F. poae* (Wang *et al.*, 2014). The inhibitory effect of nanoparticles may be due to the release of extracellular enzymes and metabolites that serve as agents for their survival when exposed to stress from toxic materials and temperature variations, as demonstrated in fungus *Trichoderma reesei* (Vahabi *et al.*, 2011). It is also observed that antifungal activity may be due to the suppression of enzymes and toxins used by the fungal pathogens for pathogenesis (Jo, 2009; Vahabi *et al.*, 2011). Also, the antifungal activity of nanoparticles could be due to the high density at which the solution could saturate and cohere to fungal hyphae and deactivate plant pathogenic fungi (Kim *et al.*, 2012).

In greenhouse and field experiments, the MgO and ZnO nanoparticles at 0.3 mM highly reduced the neck rot incidence and severity compared with the untreated control plants. Moreover, the MgO and ZnO nanoparticles at 0.3 mM highly reduced the neck rot incidence and the decline in bulb weight of onion due to infection with *B. allii* during storage. Similar results in the previous studies demonstrated that nano MgO was applied effectively as a microbicide to control *Fusarium* wilt on tomato caused by *F. oxysporum* f.sp. *lycopersici* (Parizi *et al.*, 2014), MgO and ZnO nanoparticles significantly decreased the development of damping-off and root rot severity caused by soilborne fungi *F. oxysporum* f.sp. *betae*, *Rhizoctonia solani*, and *Sclerotium rolfsii* (El-Argawy *et al*., 2017) and the nano MgO caused decreases in tobacco black shank and black root rot disease caused by *T. basicola* and *P. nicotianae* reached 36.58 and 42.35%, respectively (Chen *et al*., 2020). Therefore, based on our results, this study reports that the MgO and ZnO nanoparticles are eco-friendly alternative approaches for suppressing neck rot disease on onion in the field and storage.

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الملخص العربى

سميه بعض المواد النانوية ضد الفطر *Botrytis allii* المسبب لمرض عفن الرقبة في البصل

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في هذه الدراسة، تم الحصول على اربعه عشر عزلة فطرية من عينات بصل مصابه بأعراض مرض عفن الرقبة جمعت من مراكز مختلفة بمحافظة سوهاج. وتم التعرف عليها على أنها الفطر *Botrytis allii* Munn (اثنى عشر عزله) والفطر .*B. cinerea* Pers (عزلتين). أظهرت اختبارات القدرة المرضية التي أجريت في الصوبة أن عزلات الفطر *B. allii* كانت ممرضه وتسببت في ظهور أعراض عفن الرقبة في البصل من بين جميع العزلات الأخرى المختبرة. وكانت العزلة رقم 9 من الفطر *B. allii* عالية الضراوة حيث تسببت في 95 و 81.65٪ من حدوث المرض وشدته على التوالي. تمت دراسة تأثير جزيئات الكربون، MgO، و ZnO النانونية والتي تم اختبارها عند 0.1 و 0.2 و 0.3 ملي مول على النمو الطولي للميسليوم والكتلة الحيوية في المختبر. أظهرت النتائج أن جميع المواد النانوية قللت بشكل كبير من النمو الطولي للميسلسوم والكتلة الحيوية للفطر في جميع التركيزات المختبرة. وتسببت جزئيات MgO النانونيه عند 0.3 ملي مول في تأثير مثبط

عالي تليها جزئيات الـ ZnO النانونيه، حيث انقصت بشكل كبير من النمو الطولي للميسلسوم والكتلة الحيوية للفطر. في تجارب الصوبة والحقل، قللت الجسيمات النانوية لـ MgO و ZnO عند 0.3 ملي مول بدرجه كبيره من حدوث المرض وشدته مقارنة بالنباتات غير المعامله. علاوة على ذلك، فإن جزيئات الـ MgO و ZnO النانوية عند 0.3 ملي مول قللت بشكل كبير من حدوث عفن الرقبة، وأيضا من الانخفاض الكبير في وزن الأبصال بسبب الإصابة بالفطر أثناء التخزين. لذلك، تشير هذه الدراسة إلى أن الجزئيات النانوية للـ MgO و ZnO هي طرق بديلة صديقة للبيئة لمقاومه مرض عفن الرقبة في البصل في الحقل وأثناء التخزين.